Rashmi M G

Gene Mutation

1. Introduction mutation
2. Molecular basis of mutation
3. Spontaneous mutation
4. Induced mutation
5. Types of mutation
6. Detection of mutation
7. Role of transposable elements in Mutation
8. DNA repair mechanisms

Mutation

Genome is not a static entity. It is dynamic in nature
It is subjected to different types of heritable genetic changes

Heritable genetic change in genetic material of an organism that gives rise to alternate forms of any gene= Mutation
Process by which mutation is produced = Mutagenesis

Mutation:

- Change in chromosome number
- Chromosomal aberrations
- Change in chemistry of genes

Characteristics of Mutations:

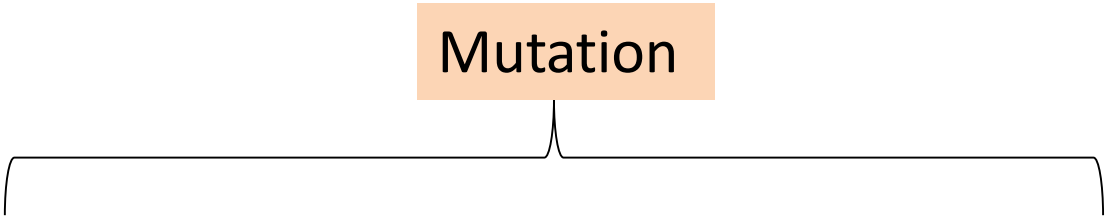
- Mutation is generally recessive (dominant mutation is less common)
- It is harmful to organisms
- It is random event (occur at any time, in any cell)
- Recurrent event (Occurs again and again)

Role of mutation:

- Source of all genetic variations
- Raw materials for evolution
- Results into formation of 2 alternate forms/ alleles for agene
- Organism would be able to evolve and adapt to environmental change

Molecular basis of gene mutation

When Watson and Crick described the double helix structure DNA and proposed its semi conservative replication based on specific base pairing to account for the accurate transmission of genetic information from generation to generation.
They also proposed a mechanism to explain spontaneous mutation



Spontaneous mutation

It occur without treatment of organism with an exogenous mutagen
Natural process
Occurs because of replication error, spontaneous lesions, transposition of transposable elements during normal growth of cell

Induced mutation

Arise due to mutagen reacted to parent DNA causing structural changes
Affects base paring capabilities of altered nucleotides

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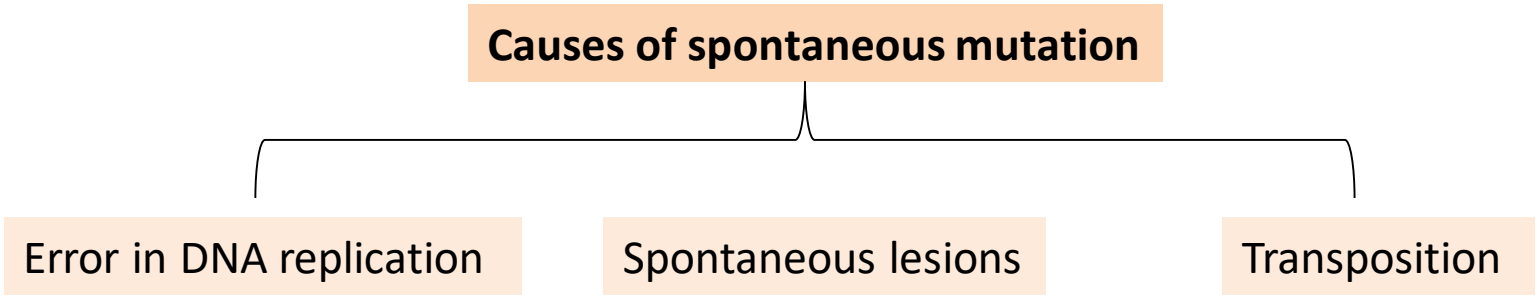
Spontaneous mutation

Spontaneous mutation

- Changes in nucleotide sequences of genes that appear to have no known cause
- No specific agents are associated with their occurrence
- Generally naturally occurring, accidental process



1.Error during DNA replication

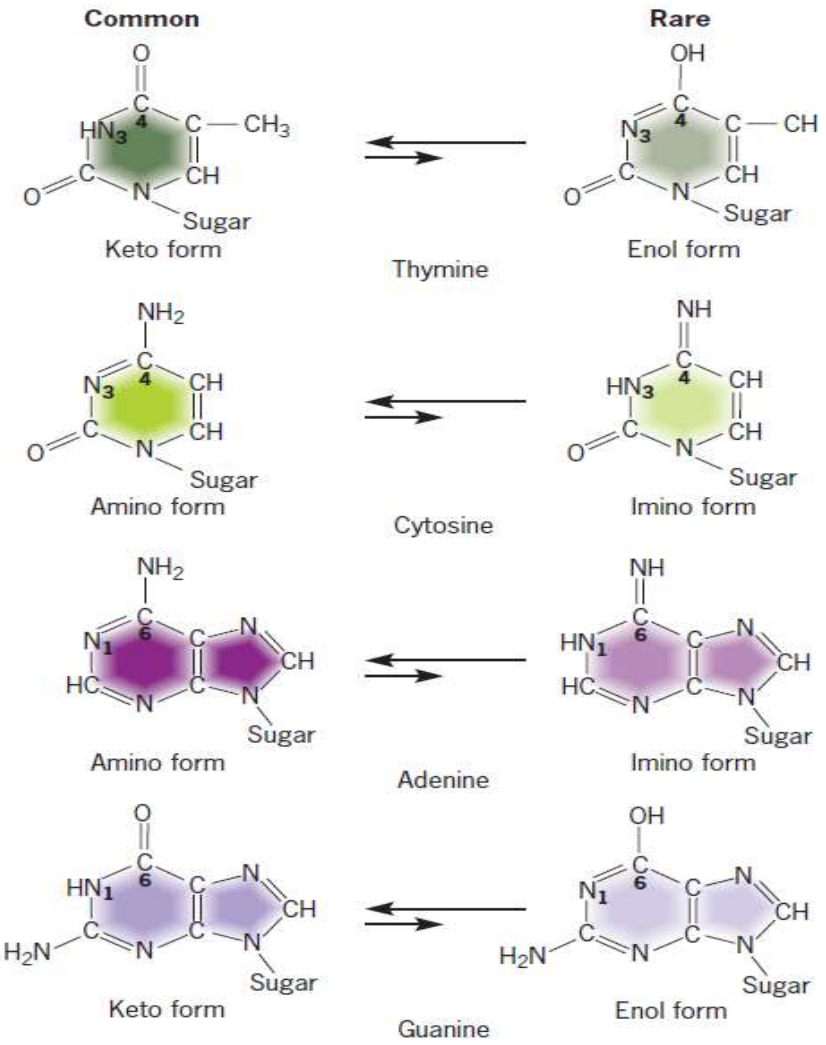
Proton shift in nitrogenous base forms one of its rare tautomeric form (**tautomeric shift**)

Stable → Tautomeric shift → **Unstable/ less stable**

Keto form
- Thymine → Enol form
- Guanine → Enol form

Amino form
- Adenine → Imino form
- Cytosine → Imino form

This results in mismatch base pairing = **A-C** , **G-T**



Source: Snustad, Simmons (2012), Principles of genetics, John Wiley& Sons, Inc, Sixth edition, Page no. 322

Error during DNA replication causes 2 types of mutation:

1. **Substitution mutation**
2. **Frameshift mutation**

1. Substitution mutation

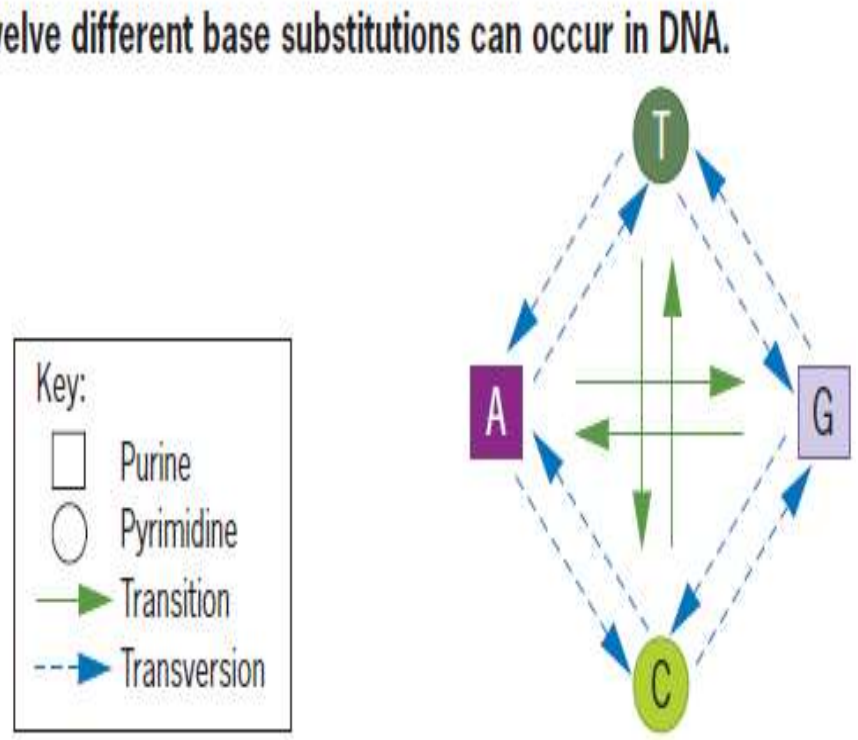
- Also called point mutation, simple mutation, single site mutation
- Results in substitution of one base pair for another
- It may cause 2 subtypes of mutation:
  a) Transition mutation
  b) Transversion mutation

Transition mutation: 1 pyrimidine is replaced by another pyrimidine; one purine is replaced by another purine

Ex: (G:C) ---> (A:T), (A:T)--->(G:C)

Transversion mutation: 1pyrimidine is replaced by another purine; 1purine is replaced by another pyrimidine

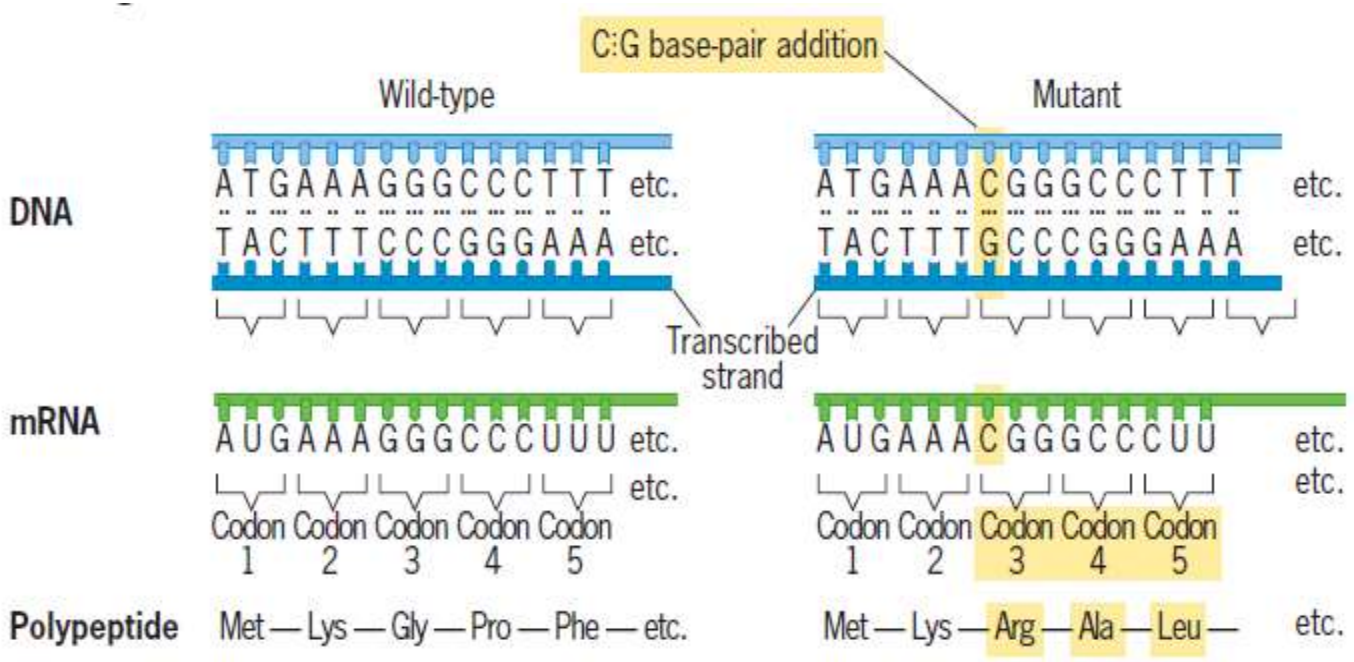
Ex: (G--->T), (G--->C), (T--->A), (T--->G), (A--->T), (A--->C), (C--->A), (C--->G)



Source: Snustad, Simmons (2012), Principles of genetics, John Wiley& Sons, Inc, Sixth edition, Page no. 324

2. Frameshift mutation

- Extra nucleotides added or some nucleotide in template not being copied
- Addition/ deletion of base pairs
- Shifts in translational reading
- Examples of Frameshift mutations are:
  - Fragile X syndrome
  - Huntington's disease
  - Kennedy syndrome myotonic dystrophy type-I
  - Spino cerebella ataxia -I



Source: Snustad, Simmons (2012), Principles of genetics, John Wiley& Sons, Inc, Sixth edition, Page no. 324

2. Spontaneous lesions

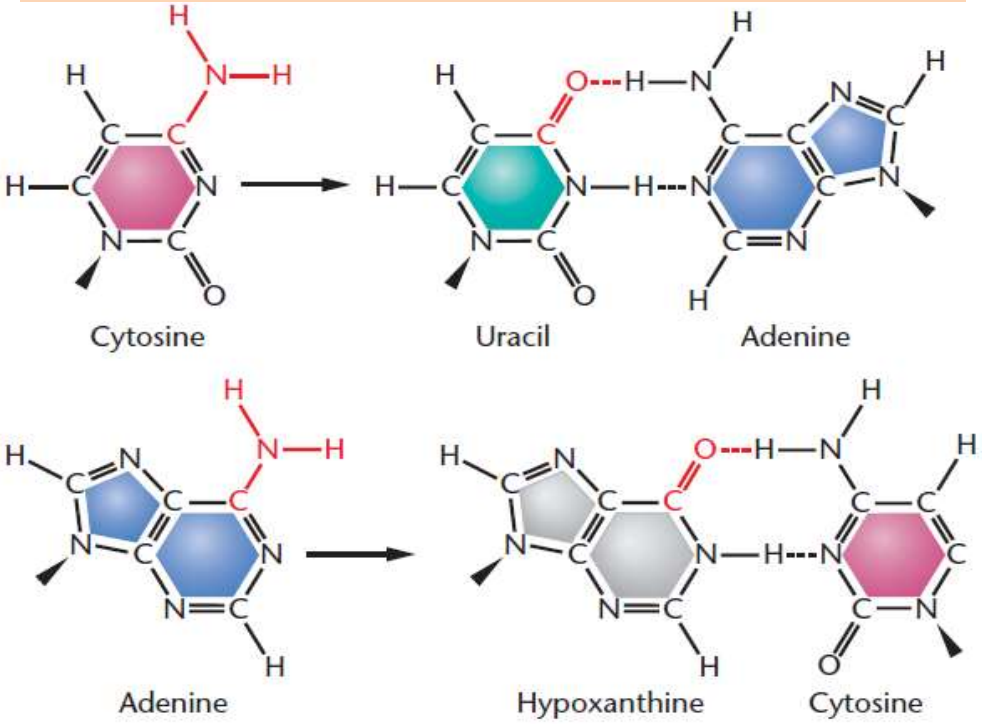
Common spontaneous lesions caused by 2 ways:

1. **Deamination**
2. **Depurination**

1. Deamination

Removal of amino acid from molecule

This results in changes in base pairing pattern



Source: Klug cummins, Spencer, Palladino (2012), Concepts of Genetics, Pearson Publication, 10th Edition, Page no.381

2. Depurination

- Loss of purine due to breaking of glycosidic bond of nucleotides in DNA
- This is common at acidic pH
- It results in formation of apurinic site

3. Depyrimidination

- Loss of pyrimidine base

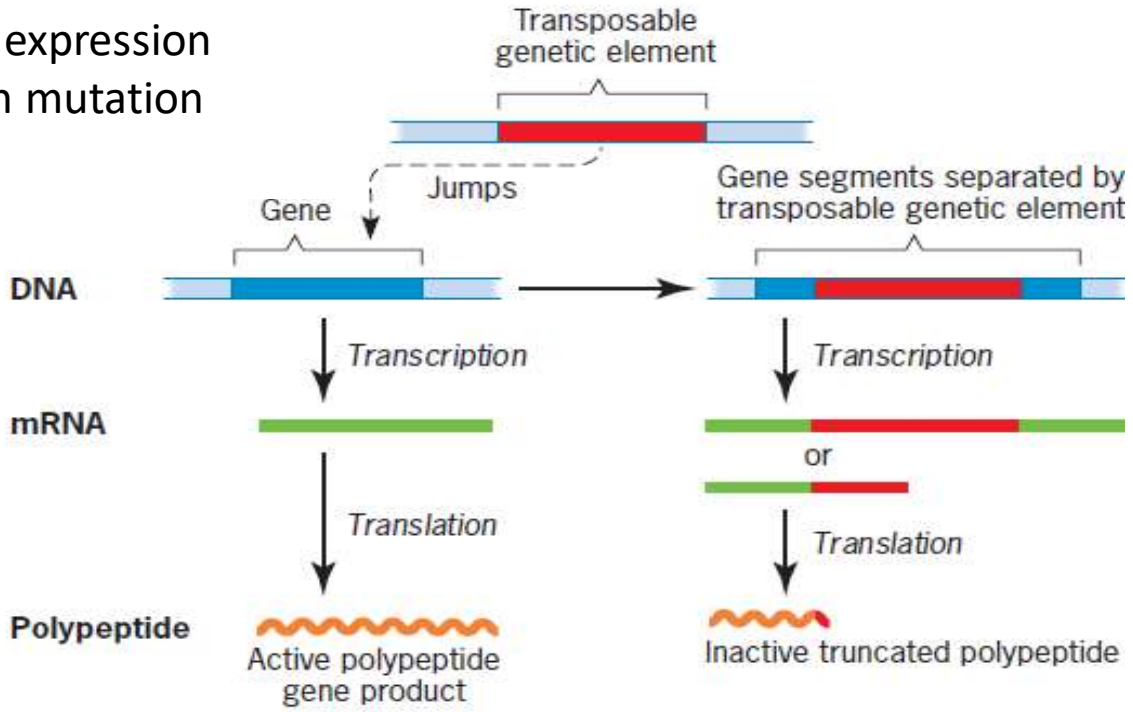
4. Oxidative damage

- Attack by reactive oxygen species
- Radicals (Superoxide radicals, hydrogen peroxide, hydroxyl radicals)
- Produce variety of products

3. Transposition

Transposons:
A DNA sequence
Able to insert itself (copy of itself) to new location in genome
The insertion of transposons is called **transposition**
The insertion of transposons near a functional gene can,

- Alter expression
- Cause loss of gene function
- Changes gene's expression
- Causes insertion mutation



Source: Snustad, Simmons (2012), Principles of genetics, John Wiley& Sons, Inc, Sixth edition, Page no. 331

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Induced mutation

Induced mutation

Mutation caused by agents, **Mutagens** like:

1. **Physical mutagens**
2. **Chemical mutagens**
3. **Biological mutagens**

Physical mutagens

1. UV radiation
2. Ionizing radiation
3. Heat

Chemical mutagens

1. Base analogs
2. Deaminating agents
3. Alkylating agents
4. Intercalating agents

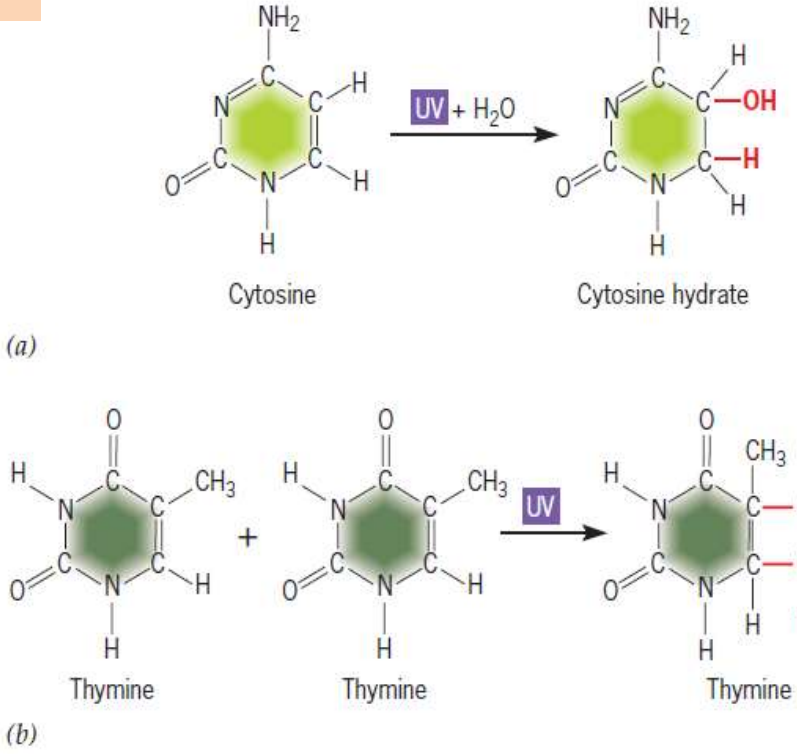
Biological mutagens

1. Viruses
2. Transposable elements

UV Radiation

Generates photoproducts in DNA

- UV- A (400-320nm), UV-B (320-280nm), UV-C (280-100nm)
- UV-260nm- Induces dimerization of adjacent pyrimidine bases
- **Adjacent pyrimidine- covalently linked by formation of 4 membered ring structure**
- Ex:Thymine presence--- **Cyclobutyl dimer (**Formation of **Pyrimidine dimer)**
- Cytosine forms **Cytosine hydrate (6-4) Lesion**



Source: Snustad, Simmons (2012), Principles of genetics, John Wiley& Sons, Inc, Sixth edition, Page no. 331

Heat

Stimulates water-induced cleavage of N-glycosidic bond

Attaches base to sugar component of nucleotide

Results in AP sites/ baseless sites (Apurinic/ Apyrimidinic)

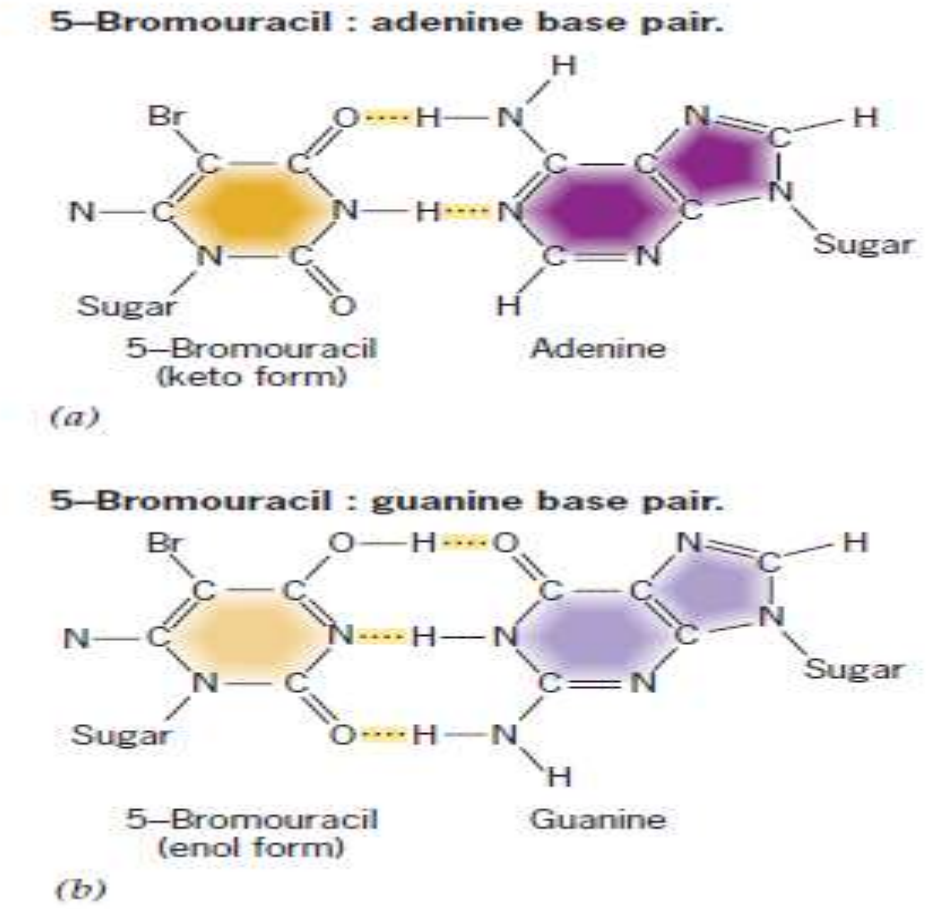
Sugar- phosphate is left unstable, rapidly degrades

Ionizing radiation

Strand breakage (acts as clastogenic factors, agents that cause chromosomal breakage)

Base analogs

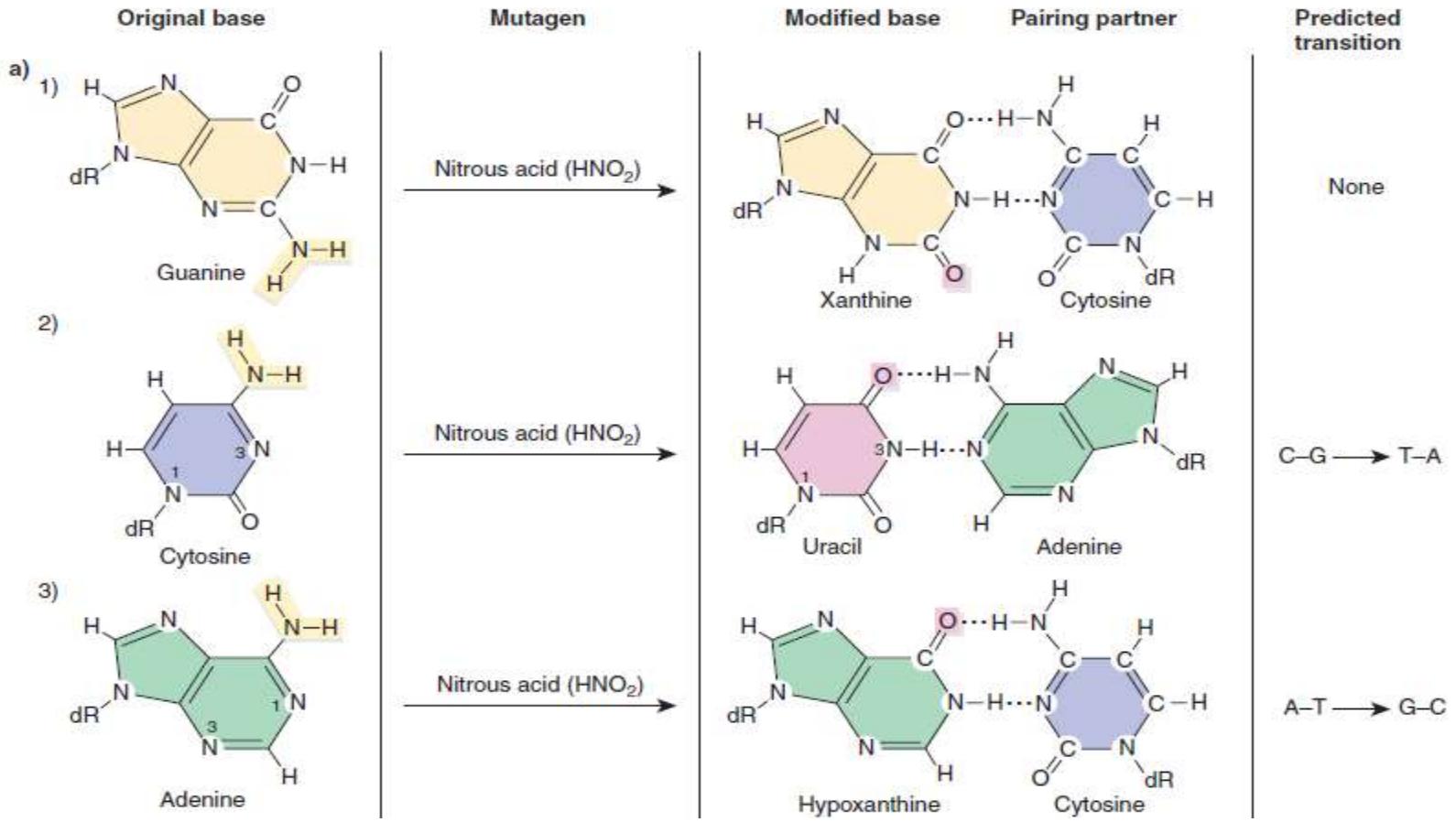
- Structurally similar to one of the bases in DNA
- **5 Bromouracil (5-BU)**
  - Structurally similar to Thymine
  - In normal keto form pairs with Adenine
  - In Enol form pairs with Guanine
  - This causes point mutation
- **2 Aminopurine (2AP)**
  - Structurally similar to Adenine
  - Amino tautomeric form pairs with Thymine
  - Imino tautomeric form pairs with cytosine
  - This induces T--->C transition during DNA replication (AT--->GC transition)



Source: Snustad, Simmons (2012), Principles of genetics, John Wiley& Sons, Inc, Sixth edition, Page no. 326

Deaminating agents

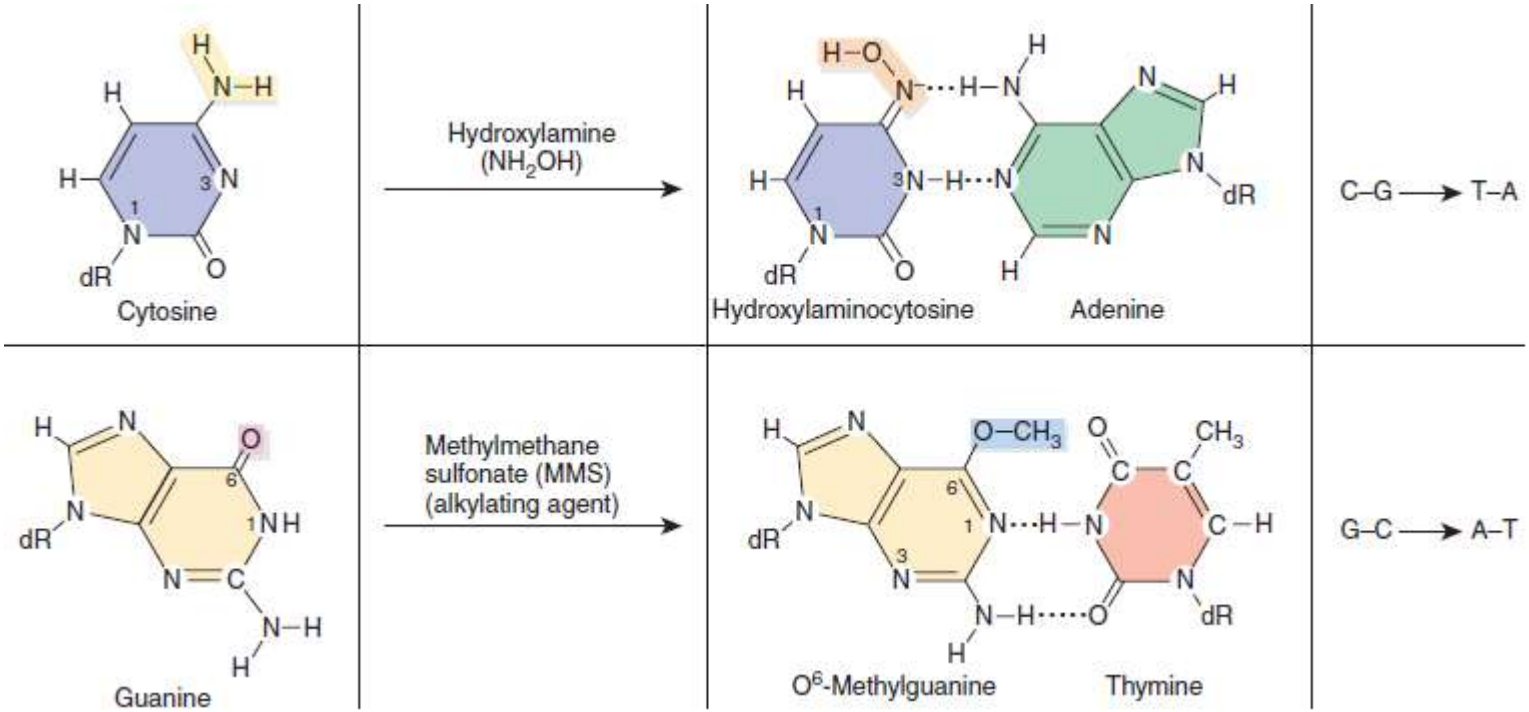
- Deaminates Adenine, Cytosine, Guanine
- (But not Thymine because no Amino group is present)
- Ex. Nitrous acid



Source: Peter J Russell, iGenetics A molecular approach, 3rd edition, Page no.142

Alkylating agents

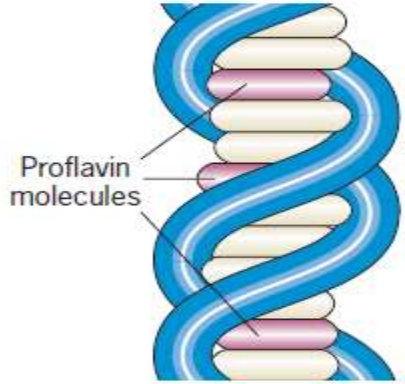
- Ethyl methyl sulfonate (EWS, Nitrogen Mustard Gas)
- Methyl methane sulfonate (MMS)
- Nitrosoguanidine (NTG)
- Dimethylnitrosamine
- This adds alkyl groups to the bases



Source: Peter J Russell, iGenetics A molecular approach, 3rd edition, Page no.142

Intercalating agents

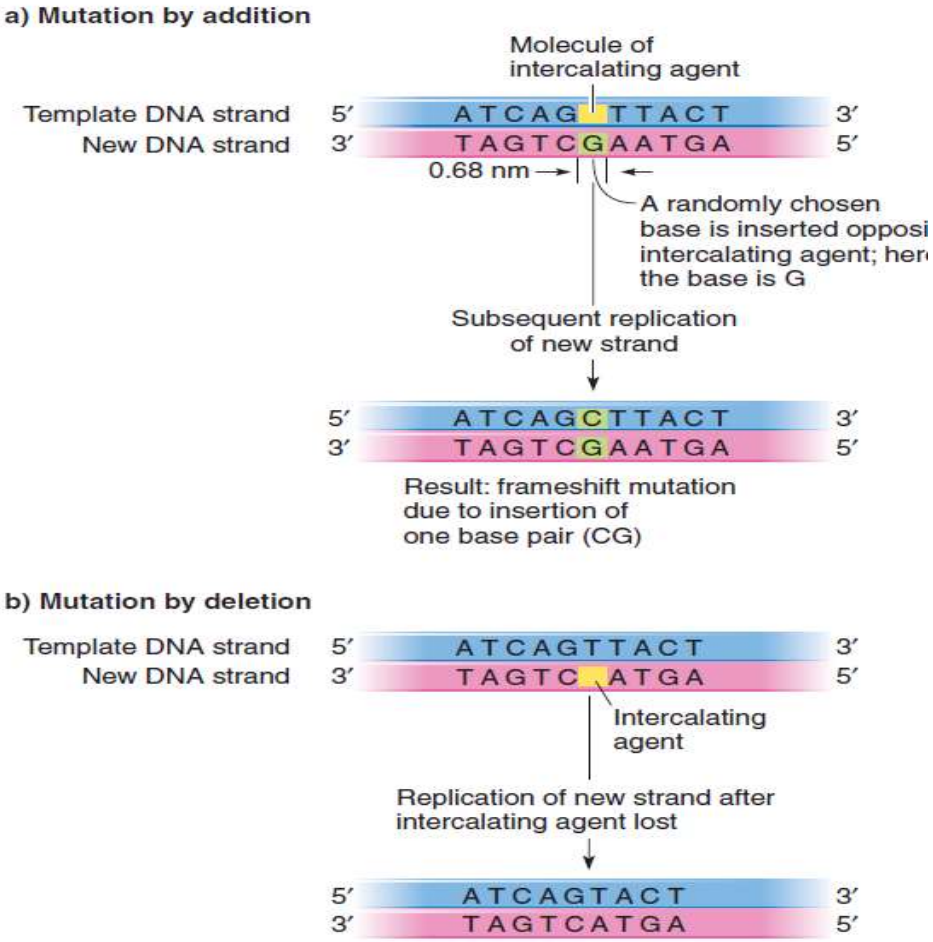
- Associated with single nucleotide pair insertion and deletion
- Proflavin, Acridine orange, Ethidium bromide, ICR-170, ICR-191
- These substances slip between base pairs in double helix, slightly unwind helix, increase distance between adjacent base pairs



■ **FIGURE 13.9** Intercalation of proflavin into the DNA double helix. X-ray diffraction studies have shown that these positively charged acridine dyes become sandwiched between the stacked base pairs.

Source: Snustad, Simmons (2012), Principles of genetics, John Wiley& Sons, Inc, Sixth edition, Page no.328

Intercalating mutations. (a) Frameshift mutation by addition, when agent inserts itself into template strand. (b) Frameshift mutation by deletion, when agent inserts itself into newly synthesizing strand.


a) Mutation by addition
Molecule of intercalating agent
Template DNA strand 5′ ATCAG TTACT 3′
New DNA strand 3′ TAGTCGAATGA 5′
0.68 nm
A randomly chosen base is inserted opposi intercalating agent; her the base is G
Subsequent replication of new strand
5′ ATCAGCTTACT 3′
3′ TAGTCGAATGA 5′
Result: frameshift mutation due to insertion of one base pair (CG)
b) Mutation by deletion
Template DNA strand 5′ ATCAGTTACT 3′
New DNA strand 3′ TAGTC ATGA 5′
Intercalating agent
Replication of new strand after intercalating agent lost
5′ ATCAGTACT 3′
3′ TAGTCATGA 5′

Source: Peter J Russell, iGenetics A molecular approach, 3rd edition, Page no.143

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Types of Mutation

Somatic and Germinal Mutation

Hereditary and Acquired Mutation

Forward and Backward Mutation

Missense and Nonsense Mutation

Loss and Gain of Function Mutation

Silent and Neutral Mutation

Suppression Mutation

Conditional Mutation

Somatic Mutation	Germinal Mutation
Mutation occurs in Somatic tissue	Mutation occurs in germinal tissue
Cannot be passed on to next generation	Arise in germline (special tissue that is set aside during development to form gametes)
	If mutant gamete participate in fertilization then it is passed on to next generation

Hereditary Mutation	Acquired Mutation
Inherited from Parent	Acquired during person's lifetime
Present throughout person's life in virtually every cell in the body	Caused by UV radiations, mistake during cell division

Missense Mutation	Nonsense Mutation
Mutation that alters the codon so that it specifies a different amino acid	Mutation that changes a codon in a gene to one of the three termination codon (UAA, UAG)
Non-synonymous mutation	Results in shortened protein
Ex. Sickle cell hemoglobin	

Forward Mutation	Back Mutation/ Reversion Mutation	
Mutation changing phenotype from Wild Type to Mutant Type	Mutation changing phenotype from Mutant Type to Wild Type	
	True Reversion	**Equivalent Reversion**
	Mutation at same site in gene as original mutation	Mutation does not generate original condition at gene level
	Restores wild type nucleotide sequence Exact reversal of original mutation	Restores wild type phenotype due to formation of synonymous codon
	Change in same codon and same nucleotide	Change in same codon but different nucleotide

Loss of Function Mutation	Gain of Function Mutation
Null mutation	
The resulted product is reduced/ have no function	The resulted product of increased/ have new function
Found to be recessive (some are dominant)	Cause dominant phenotype
Ex. In Heterozygote's, The recessive allele= loss of function	Because presence of normal allele does not prevent mutant allele from behaving abnormally

Silent Mutation	Neutral Mutation
Mutation without apparent effect (no detectable change) If mutation in which new codon specifying same Amino acid as unmutated codon= silent / synonymous Ex. Change of ACG--->CGG Both codes for Arginine	Mutation where codon specifies different but functionally equivalent amino acid and not alter protein function Ex. Change of AAA--->AGA Changing basic lysine to basic arginine

Suppression Mutation	
Restores function lost by First Mutation	
Suppress effects of First Mutation	
Can be,	
Intragenic	**Intergenic**
Restores activity of mutant protein by many means	Restores activity of mutant protein
Occur at distinct sites within same gene	Do not occur in same gene
	Provide another gene product to take its place

Conditional Mutation
Expresses mutant phenotype only in certain environmental condition= **Restrictive Condition**
But produce wild type phenotype in some different environmental condition= **Permissive condition**
Ex. Some conditional mutants are temperature- sensitive mutant which give wild type phenotype at low temperature but exhibit mutant phenotype at high temperature

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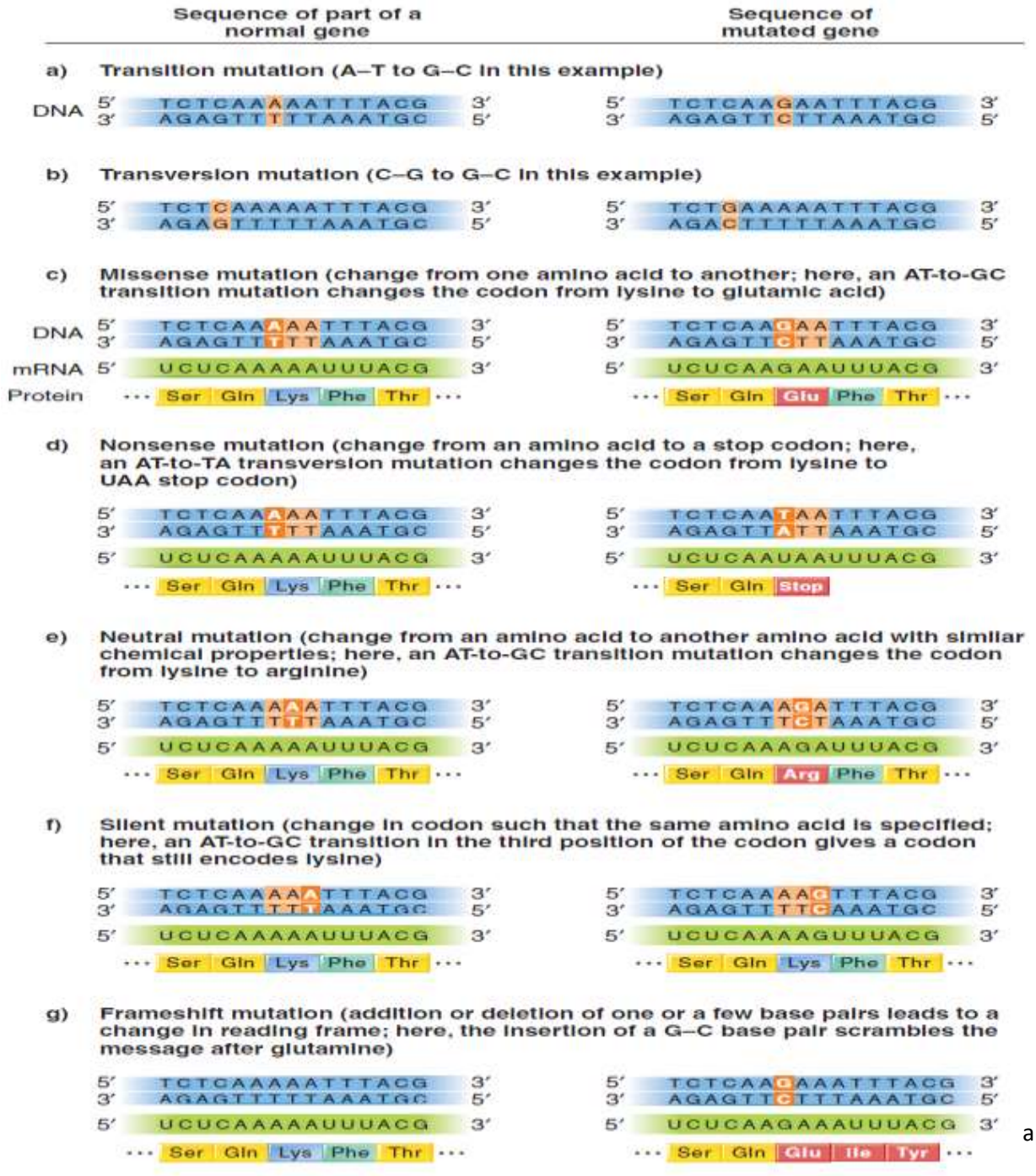
Sequence of part of a normal gene
Sequence of mutated gene
a) Transition mutation (A–T to G–C in this example)
DNA
5′ TCTCAAAAATTTACG 3′
3′ AGAGTTTTTAAATGC 5′
5′ TCTCAAGAATTTACG 3′
3′ AGAGTTCTTAAATGC 5′
b) Transversion mutation (C–G to G–C in this example)
5′ TCTCAAAAATTTACG 3′
3′ AGAGTTTTTAAATGC 5′
5′ TCTGAAAAATTTACG 3′
3′ AGACTTTTTAAATGC 5′
c) Missense mutation (change from one amino acid to another; here, an AT-to-GC transition mutation changes the codon from lysine to glutamic acid)
DNA
mRNA
Protein
5′ UCUCAAAAAUUUACG 3′
··· Ser Gln Lys Phe Thr ···
5′ UCUCAAGAAUUUACG 3′
··· Ser Gln Glu Phe Thr ···
d) Nonsense mutation (change from an amino acid to a stop codon; here, an AT-to-TA transversion mutation changes the codon from lysine to UAA stop codon)
5′ UCUCAAAAAUUUACG 3′
··· Ser Gln Lys Phe Thr ···
5′ UCUCAAUAAUUUACG 3′
··· Ser Gln Stop
e) Neutral mutation (change from an amino acid to another amino acid with similar chemical properties; here, an AT-to-GC transition mutation changes the codon from lysine to arginine)
5′ UCUCAAAAAUUUACG 3′
··· Ser Gln Lys Phe Thr ···
5′ UCUCAAAGAUUUACG 3′
··· Ser Gln Arg Phe Thr ···
f) Silent mutation (change in codon such that the same amino acid is specified; here, an AT-to-GC transition in the third position of the codon gives a codon that still encodes lysine)
5′ UCUCAAAAAUUUACG 3′
··· Ser Gln Lys Phe Thr ···
5′ UCUCAAAAGUUUACG 3′
··· Ser Gln Lys Phe Thr ···
g) Frameshift mutation (addition or deletion of one or a few base pairs leads to a change in reading frame; here, the insertion of a G–C base pair scrambles the message after glutamine)
5′ UCUCAAAAAUUUACG 3′
··· Ser Gln Lys Phe Thr ···
5′ UCUCAAGAAAUUUACG 3′
··· Ser Gln Glu Ile Tyr ···

Source: Peter J Russell, iGenetics A molecular approach, 3rd edition, Page no.133

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Detection of Mutation

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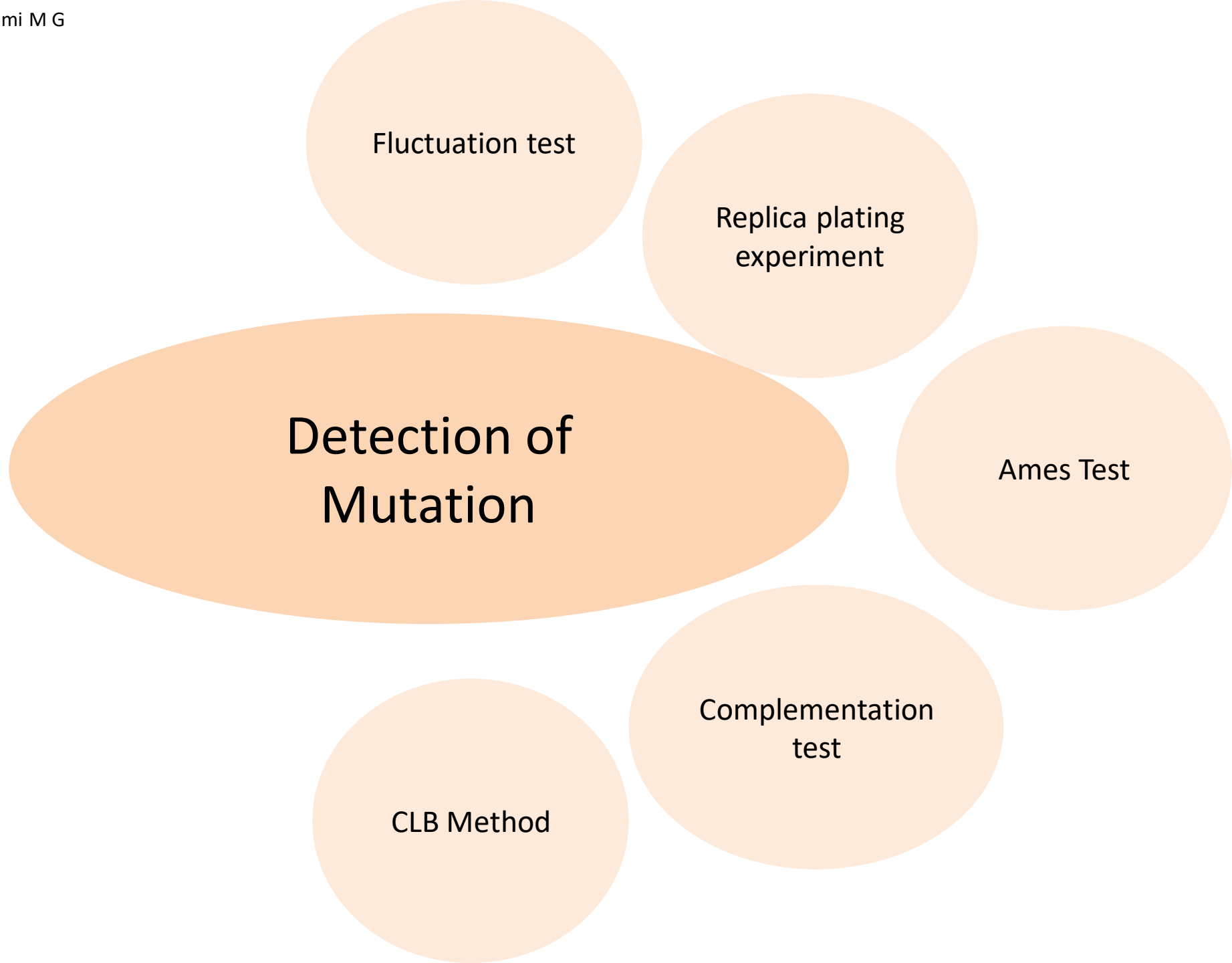
Fluctuation test
Replica plating experiment
Detection of Mutation
Ames Test
Complementation test
CLB Method

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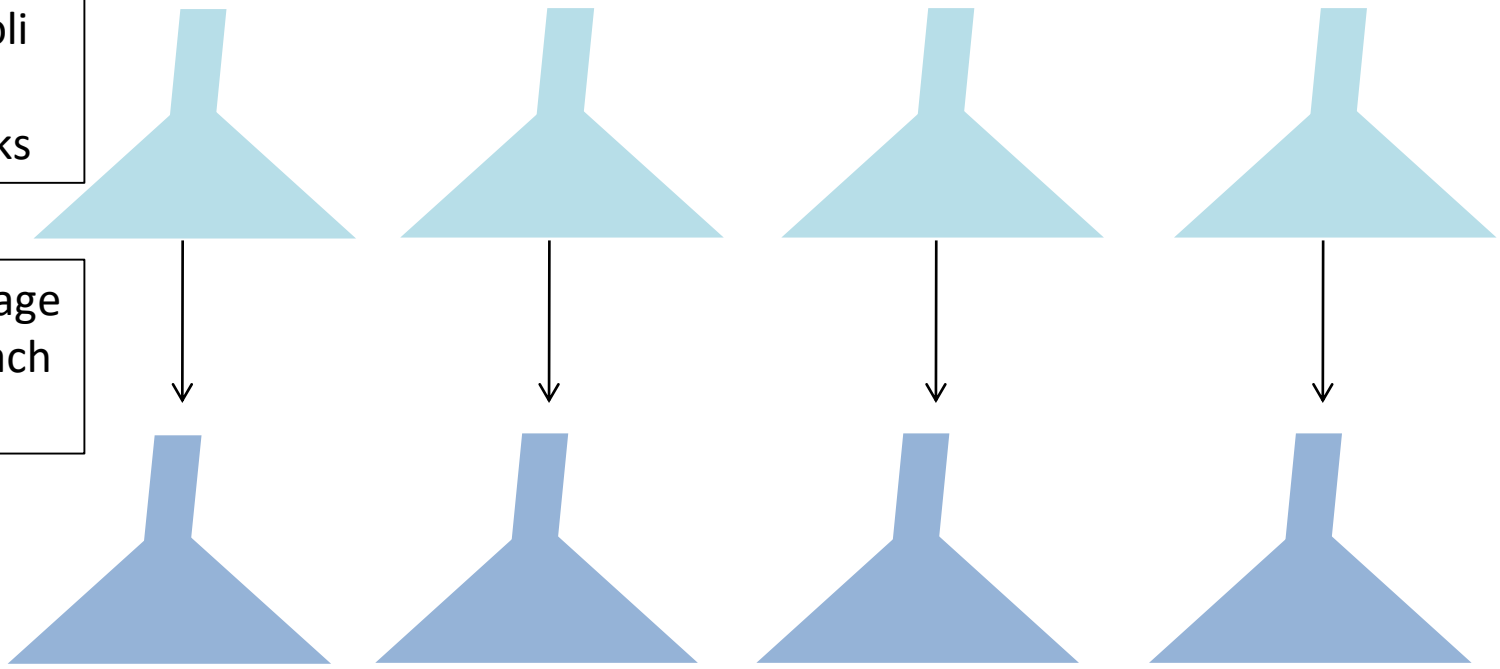
Fluctuation test

- Luria and Delbruck (1943)
- To determine Randomness of mutation in Bacteria

Series of E coli cultures in different flasks

T1 bacteriophage is added to each one



Most Bacteria killed by phage
Few T1 resistant mutants= survived

- Measured the number of mutants resistant to bacteriophage T1
- If mutant occur after culture is exposed to phage = little variation should occur among cultures in number of mutants
- If mutants arise at random during no selective growth of cells= Each culture would contain different number of resistant mutant

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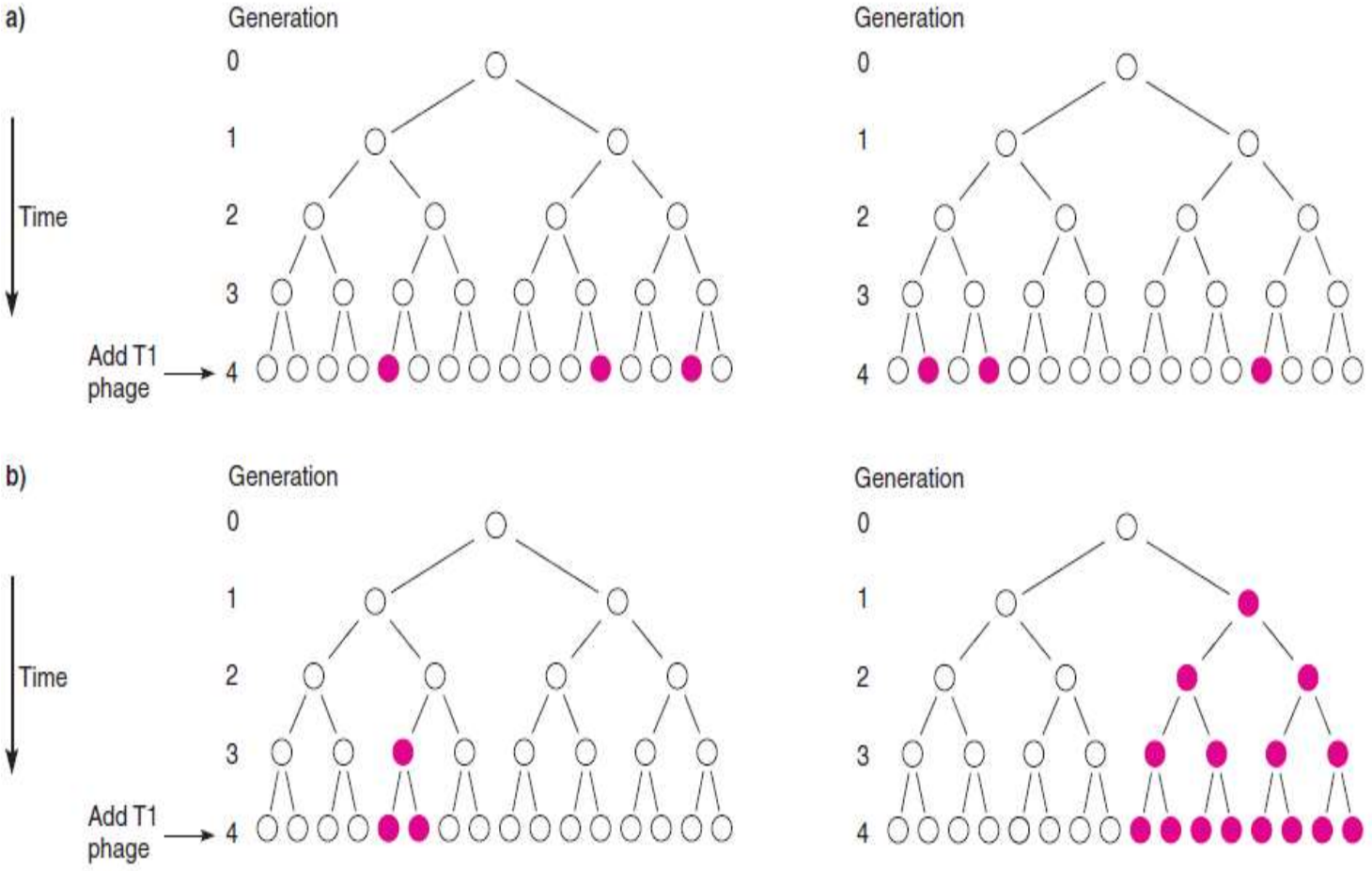
Representation of a dividing population of T1 phage-sensitive wild-type *E. coli*. At generation 4, T1 phage is added. (a) If the adaptation theory is correct, cells mutate only when T1 phage is added, so the proportions of resistant cells in duplicate cultures are the same. (b) If the mutation theory is correct, cells mutate independently of when T1 phage is added, so the proportions of resistant cells in duplicate cultures are different. *Left:* If one cell mutates to become resistant to T1 phage infection at generation 3, then 2 of the 16 cells at generation 4 are resistant to T1. *Right:* If one cell mutates to become resistant to T1 phage infection at generation 1, then 8 of the 16 cells at generation 4 are resistant to T1.

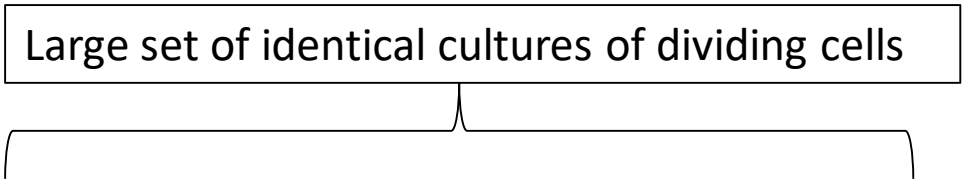


Source: Peter J Russell, iGenetics A molecular approach, 3rd edition, Page no.132

Result interpretation:

Numbers- depend on how early during growth of population the mutation occurred Mutation,

- During early generation- large clone of mutant cells
- Late mutation- Few mutant cells



Few cultures	**Majority cultures**
Have large number of mutants	Have none/ few mutants
Mutation happened in early generation	Mutation happened in late stage

Conclusion:

1. This test measures degree of fluctuation in number of mutants found in replicate cultures
2. This determines mutation rates during non selective growth
3. Mutation occurs before selection

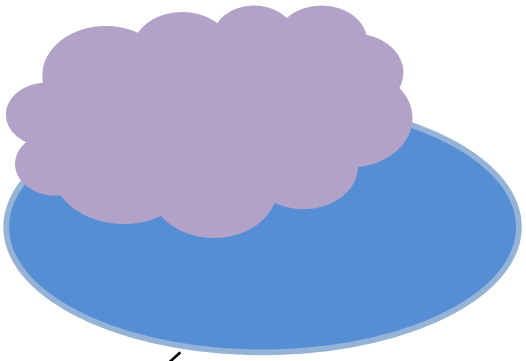
Rashmi M G

Replica plating experiment

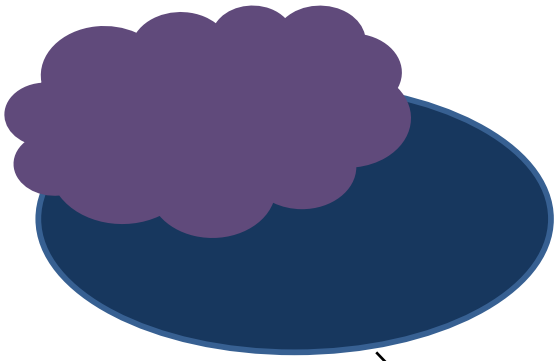
- Joshua and Esther Lederburg (1952)
- Resistant cells are selected by environmental agent rather than produced by it (Non adaptive nature of mutation)

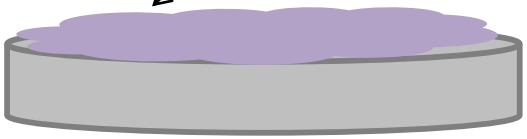
Population of bacteria
Plated on non selective medium
"Master plate"

Population of bacteria
Plated on selective medium
(Contains T1 phage)



Sterile piece of velvet pressed down lightly on surface of plates
Velvet picked up cells wherever there was a colony





Cells clinging to velvet are inoculated onto replica plates in same relative positions as those of colonies on original plate

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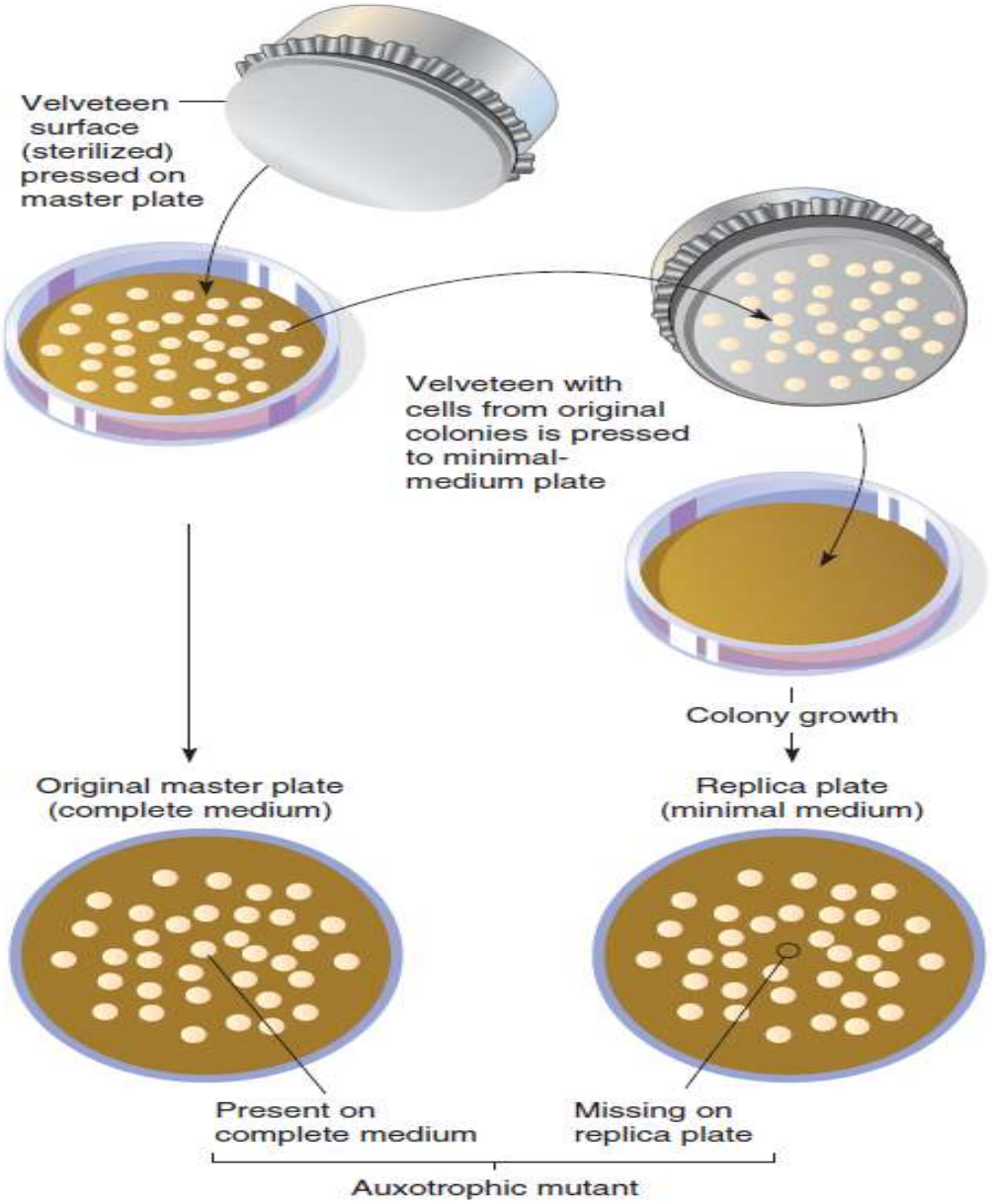
Replica-plating technique to screen for mutant strains of a colony-forming microorganism.


Velveteen surface (sterilized) pressed on master plate
Velveteen with cells from original colonies is pressed to minimal-medium plate
Colony growth
Original master plate (complete medium)
Replica plate (minimal medium)
Present on complete medium
Missing on replica plate
Auxotrophic mutant

Source: Peter J Russell, iGenetics A molecular approach, 3rd edition, Page no.145

Result interpretation:

- Rare Tom^r Mutant colonies- found on Replica plate
- Multiple Replica plates- showed identical patterns of resistant colonies
- If mutation has occurred after exposure to selective agents- Patterns for each plate would have been random as mutations themselves

Conclusion:

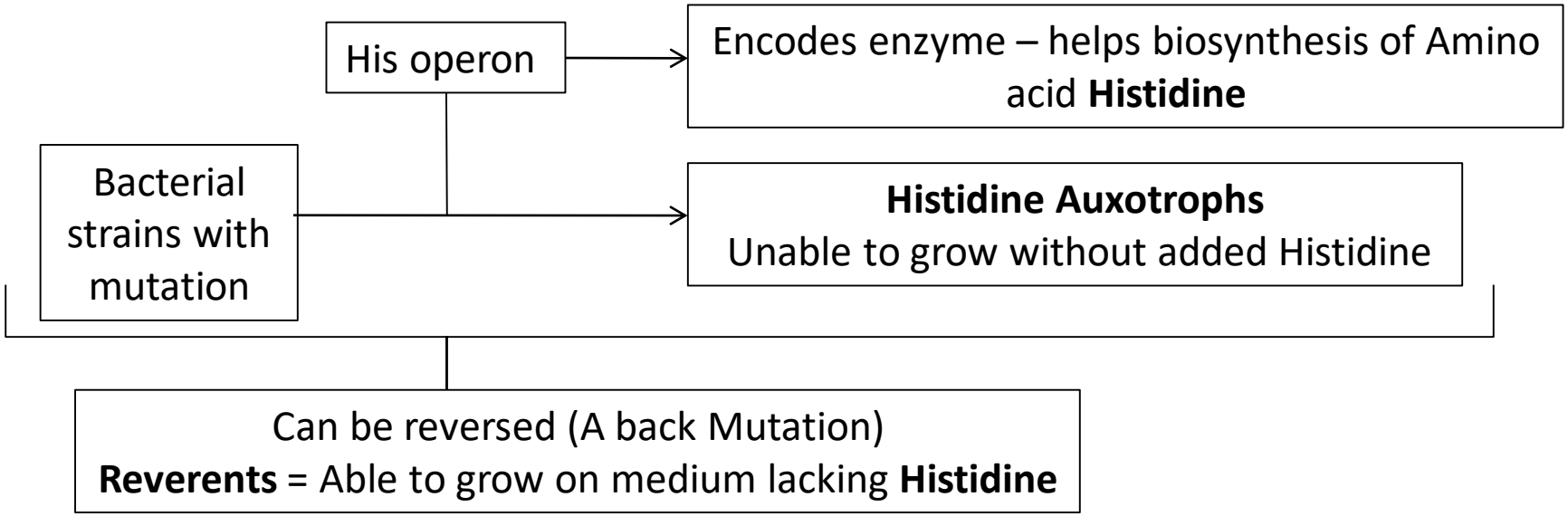
1. This test is useful in screening for mutants that fail to grow under selective regime
2. Position of absent colony on replica plate is used to retrieve the mutant from master
3. Retains original set of strains on master plate

Rashmi M G

Ames test

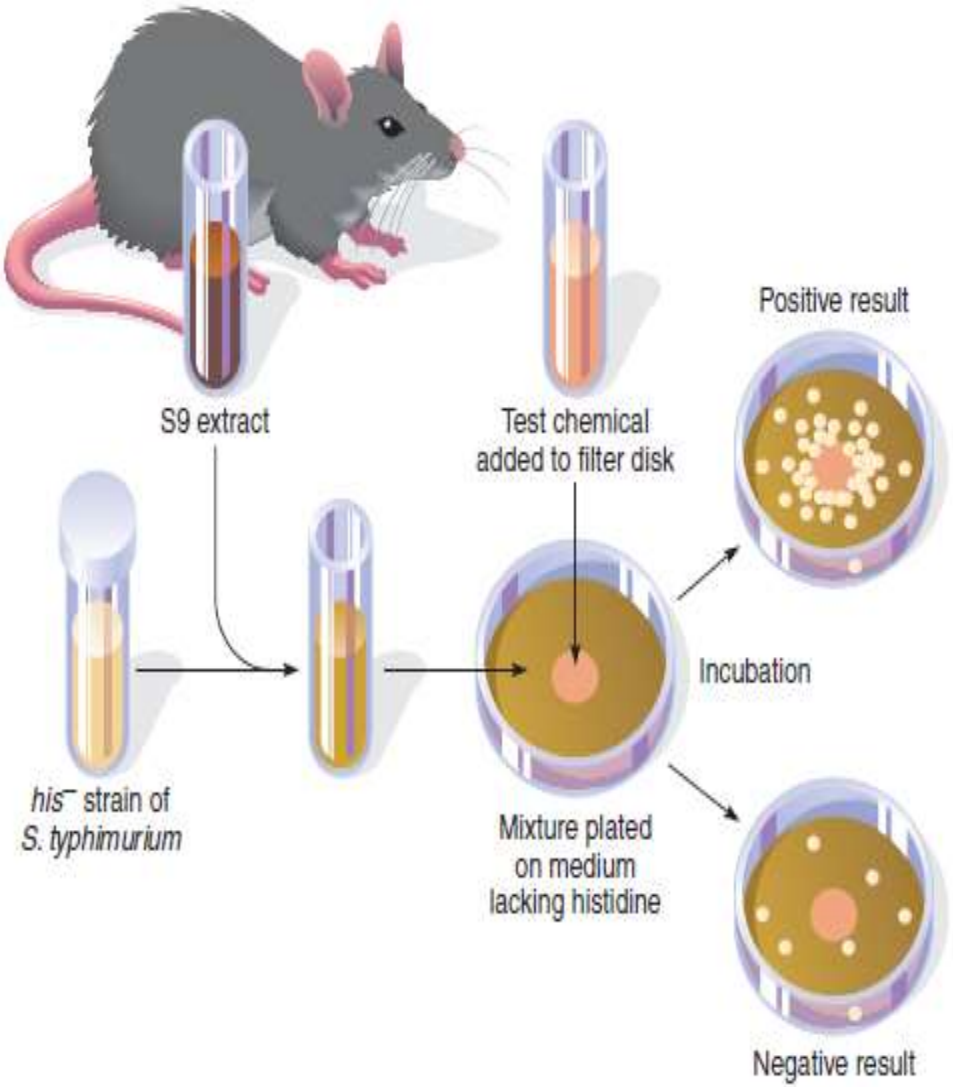
- Bruce Ames
- To test chemicals for their cancer causing properties
- Use of this test, based on assumption that any substance that is mutagenic may also turn out to be carcinogen that is to cause cancer

Principle: Reversion of mutations in histidine (His) operon in genetically altered tester strains of bacterium *Salmonella typhimurium*


His operon
Encodes enzyme – helps biosynthesis of Amino acid **Histidine**
Bacterial strains with mutation
Histidine Auxotrophs
Unable to grow without added Histidine
Can be reversed (A back Mutation)
Reverents = Able to grow on medium lacking **Histidine**

Tester strains constructed,
To have both Frameshift and point mutations in genes required to synthesize histidine

Allows for detection of mutagens acting via different mechanisms



The Ames test for assaying the potential mutagenicity of chemicals.

Source: Peter J Russell, iGenetics A molecular approach, 3rd edition, Page no.144

Importance:

- To detect mutagens that work directly to alter DNA
- To detect promutagenic agents
- In humans,
  - Chemicals- Promutagens (agents that must be activated to become true mutagens)
  - Activation involved chemical modification which occurs in Liver as a consequence of normal liver activity on unusual substances
- In Bacteria, (Salmonella typhimurium)
  - No production of enzymes required for activation of Promutagens
  - So Promutagens not be detected by Ames test
  - Mixing of test compound+ enzyme from Rat Liver (which activates Promutagens)
  - Ames test detects

Complementation test

- If 2 recessive mutation arise independently and both have same phenotype (**How to know whether the both mutation of same gene or different gene**)
- This test allows us to determine whether 2 mutations, both of which produce a similar phenotype are in same gene (i.e. whether they are alleles/ represent mutations in separate genes, whose proteins are involved in same function)

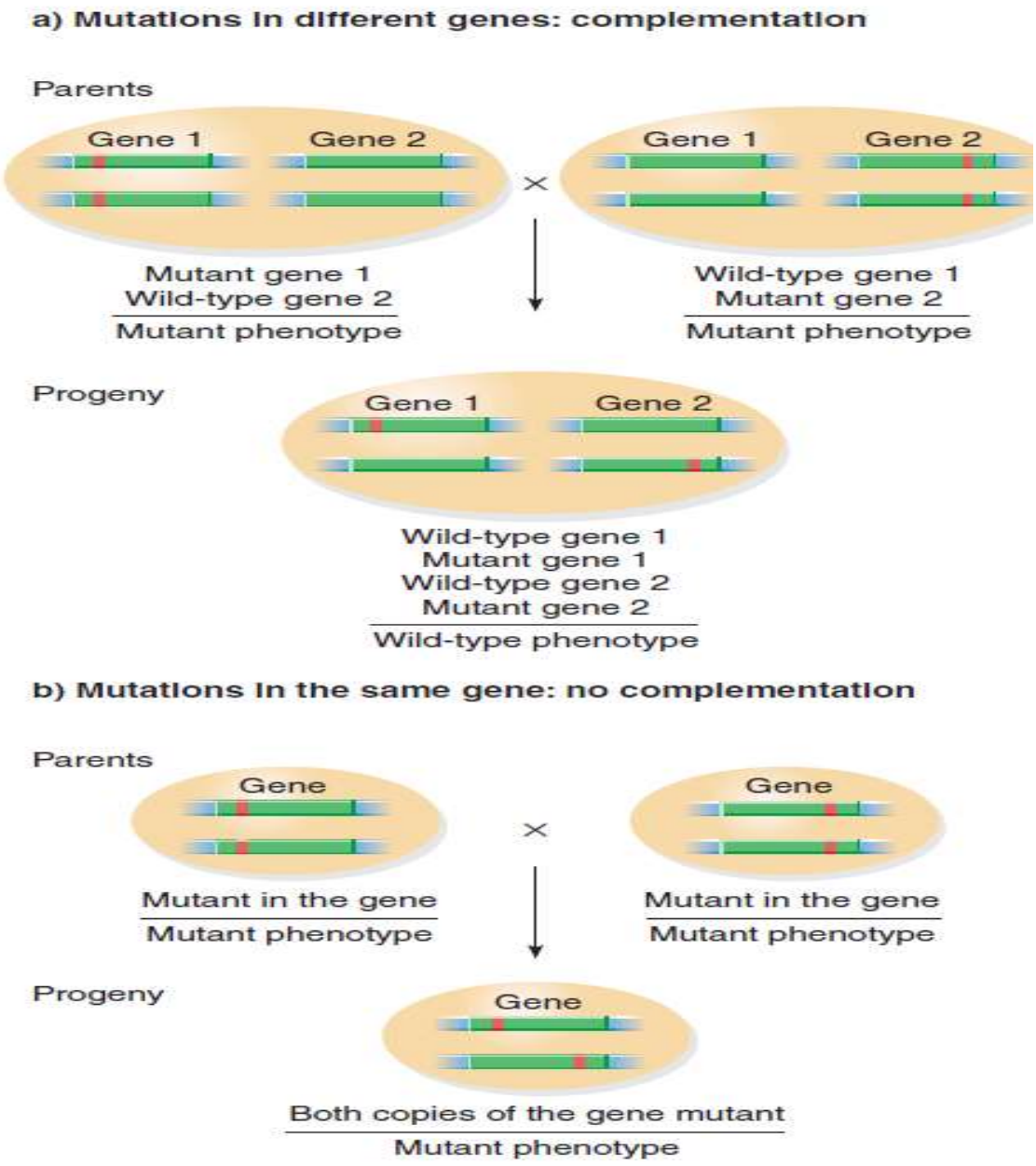
Complementation occurs,

- Only if mutations are in **different genes**
- When 2 strains of an organism with different homozygous recessive mutations that produce same phenotype produce offspring with wild type phenotype when mated/ crossed

Complementation test of Allelism (Allelism test)

- In diploid organism, is done by intercrossing homozygous recessive mutants 2 at a time
- Observing whether/ not the progeny have a wild type phenotype

Complementation test to determine whether two mutations resulting in the same phenotype are in the same or different genes.



Source: Peter J Russell, iGenetics A molecular approach, 3rd edition, Page no.377

Result Prediction:

If 2 recessive mutation,

a) **In separate genes and are not alleles of one another**
   - Then following the cross, all F1 progeny are heterozygous for both genes
   - Normal products of both gene are produced
   - Complementation occurred

b) **In same genes and are alleles of one another**
   - F1 is homozygous for 2 mutant alleles
   - No normal product of gene is produced
   - Complementation does not occur

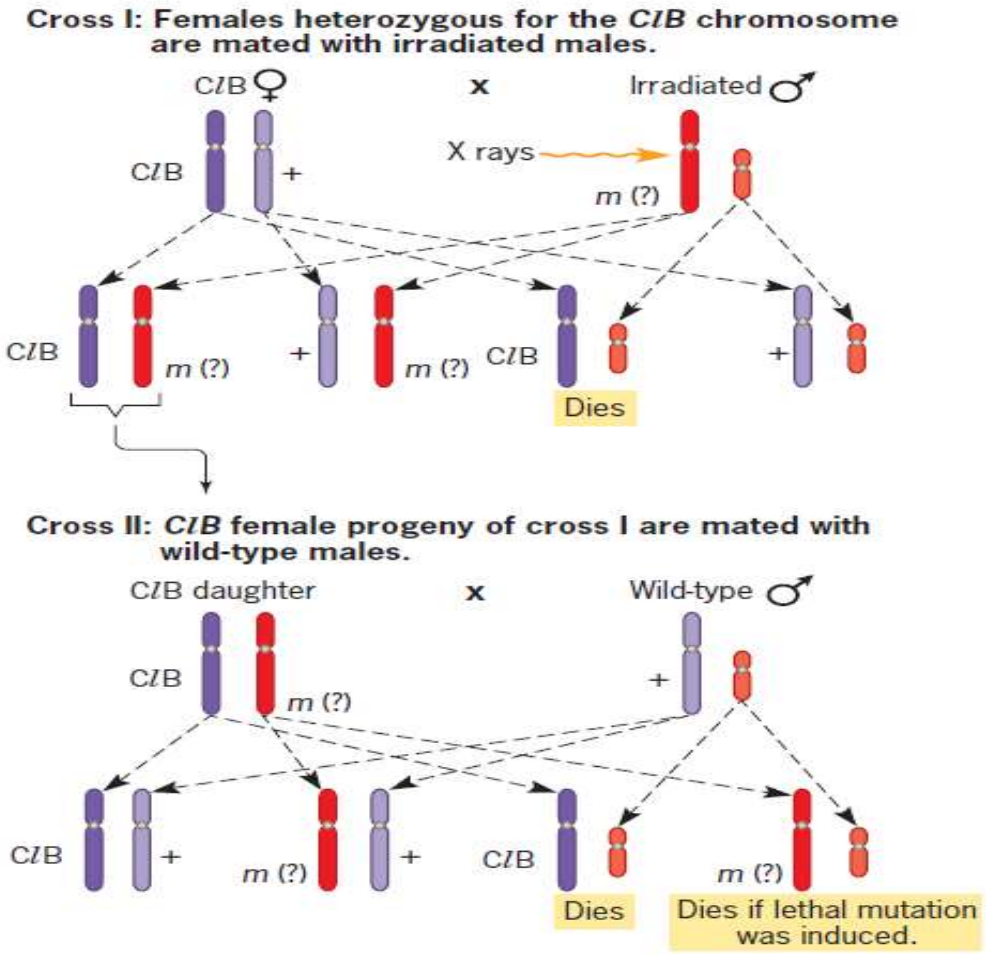
CLB Method

- H J Muller (1927)
- Demonstration of mutagenicity of X rays become possible because he developed a simple and accurate technique that could be used to identify lethal mutations on X chromosome of *Drosophila*

Female heterozygous for a normal X chromosome: altered X chromosome (Clb chromosome)

Clb chromosome has 3 components:

1. C- crossover suppressor- refers to long inversion that suppresses recombination between Clb chromosome and structurally normal X chromosome in heterozygous females
2. L- recessive lethal mutation on the Clb chromosome
3. B- mutation that causes the bar-eye phenotype



■ **FIGURE 13.4** The *ClB* technique used by Muller to detect X-linked recessive lethal mutations (*m*) in *Drosophila*. The mating shown in Cross II will produce only female progeny if an X-linked recessive lethal is present on the irradiated X chromosome. One-third of the progeny produced from Cross II will be males if there is no recessive lethal on the irradiated X chromosome. Thus, scoring for lethal mutations simply involves screening the progeny of Cross II for the presence or absence of males.

Source: Snustad, Simmons (2012), Principles of genetics, John Wiley& Sons, Inc, Sixth edition, Page no. 325

- Male flies (X ray irradiated) and crossed with Clb/+ Females
- All bar eyed daughters of this mating carried- Clb chromosome of female parent and the irradiated X chromosome of male parent
- These bar eyed daughters were then mated individually (in separate cultures) with wild type males

Result interpretation:

- If irradiated X chromosome carried by a bar eyed daughter had acquired an X-linked lethal, all the progeny of the mating would be female
- Males hemizygous for the Clb chromosome would die because of the recessive lethal (l) this chromosome carries
- Males hemizygous for the irradiated X chromosome would die if a recessive lethal had been induced on it.
- Mating of bar eyed daughters carrying an irradiated X chromosome in which no lethal mutation had been induced would produce female and male progeny in ration 2:1 (only males with Clb chromosome will die)

Importance:

To demonstrate 150 fold increase in the frequency of X linked lethals after treating male flies with X rays

Rashmi M G

Role of transposons in mutation

Transposons

Transposable elements/ Transposons/ Jumping genes:

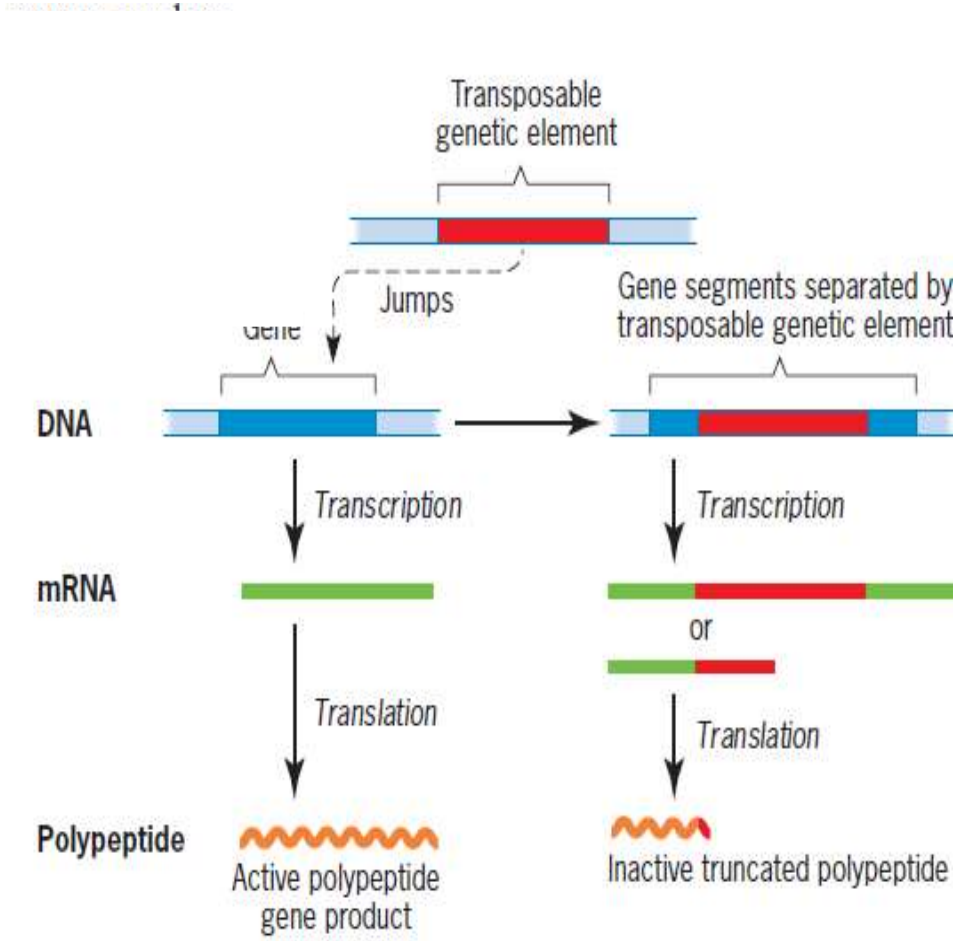
- Move or transpose within and between chromosomes
- Inserts themselves into various locations within the genome

General features:

- Normal
- Ubiquitous components of genome
- Present in both prokaryotes and eukaryotes

2 classes:

1. Transposable elements in Prokaryotes
2. Transposable elements in Eukaryotes



Source: Snustad, Simmons (2012), Principles of genetics, John Wiley& Sons, Inc, Sixth edition, Page no. 331

Transposable elements in Prokaryotes

Transposable elements move to new position on the same chromosome or onto plasmids or phage chromosome

Transposable elements in Eukaryotes

Transposable elements may move to new position within same chromosome or to different chromosome

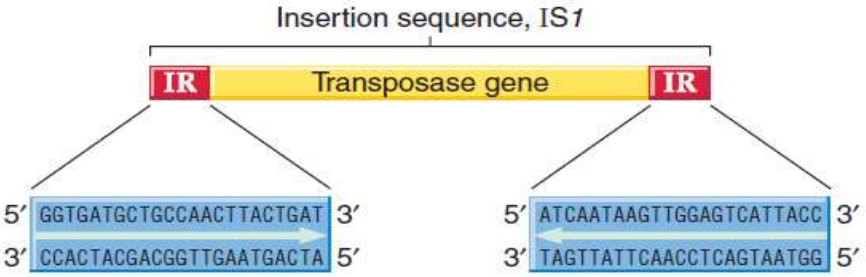
Role of Transposons in mutation

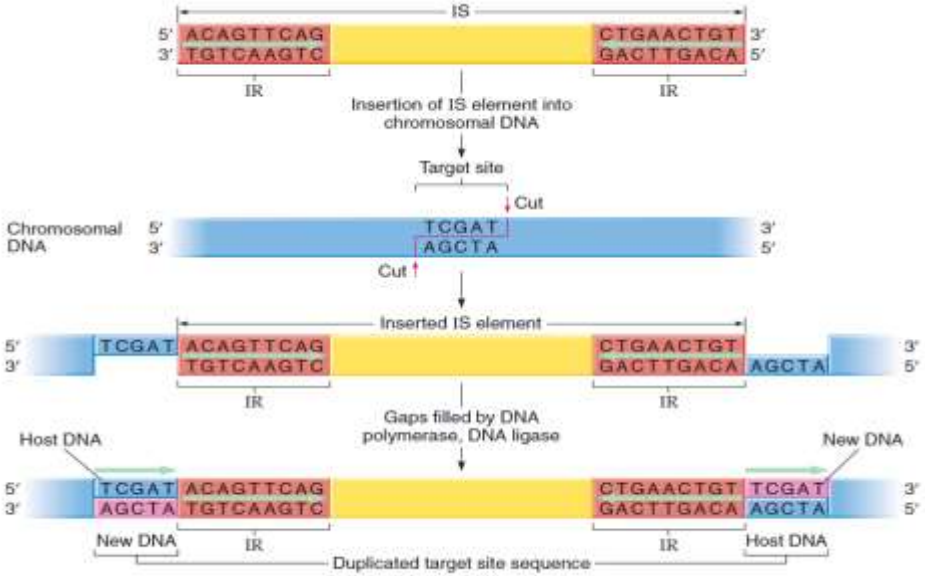
- They insert into new chromosome locations with which they have no sequence homology
- Therefore called **non-homologous recombination**
- They cause genetic changes
- They can produce mutations by inserting into genes (Process called **insertional mutagenesis**)
- Can increase/ decrease gene expression by inserting into gene regulatory sequences (such as disrupting promoter function or stimulating a gene's expression through the activity of promoters on the element
- Can produce various kinds of chromosomal mutations through the mechanics of transposition

Examples of Transposable elements in Bacteria

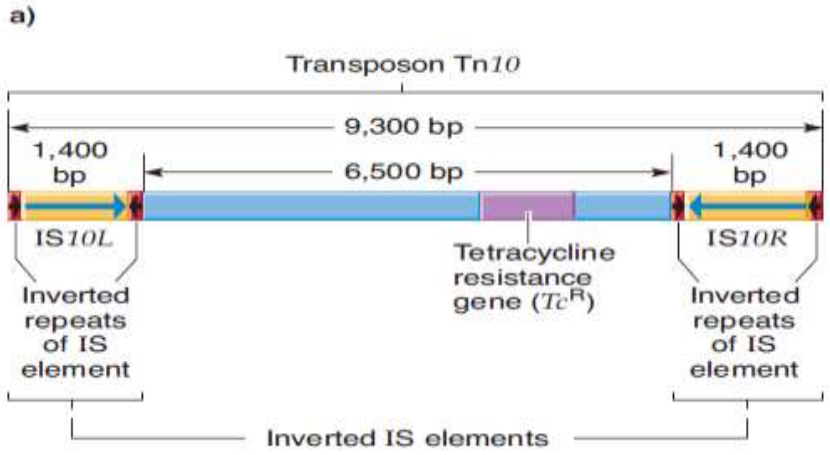
1. Insertion sequence Elements (IS)

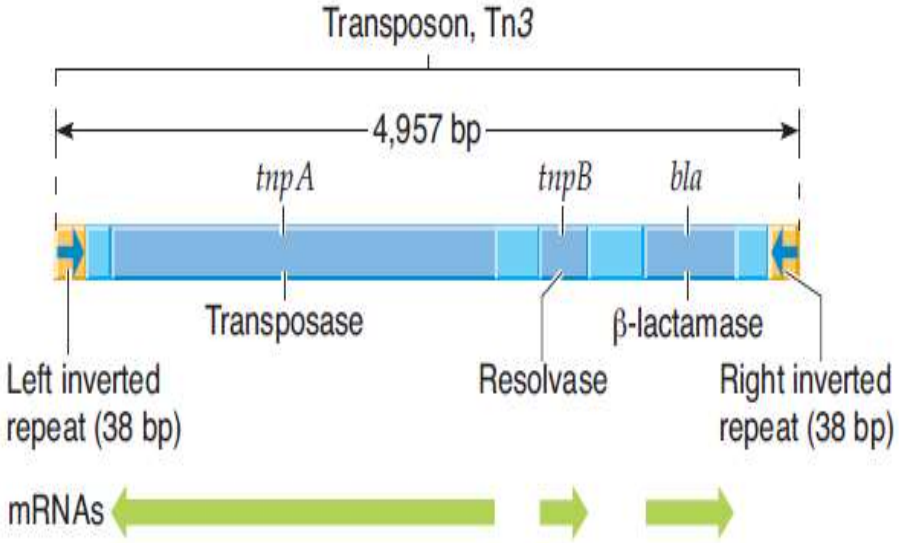
The insertion sequence (IS) transposable element IS*1*. The 768-bp IS element has inverted repeat (IR) sequences at the ends. Shown below the element are the sequences for the 23-bp terminal inverted repeats (IR).





2. Transposons (Tn)





Mc Clintock's Experiment

Barbara Mc Clintock did a series of elegant genetic experiments with Zea mays (Corn) that led her to hypothesize the existence of what she called "controlling elements" which modify/ suppress gene activity in corn and are mobile in the genome

These controlling elements were nothing but Transposable elements

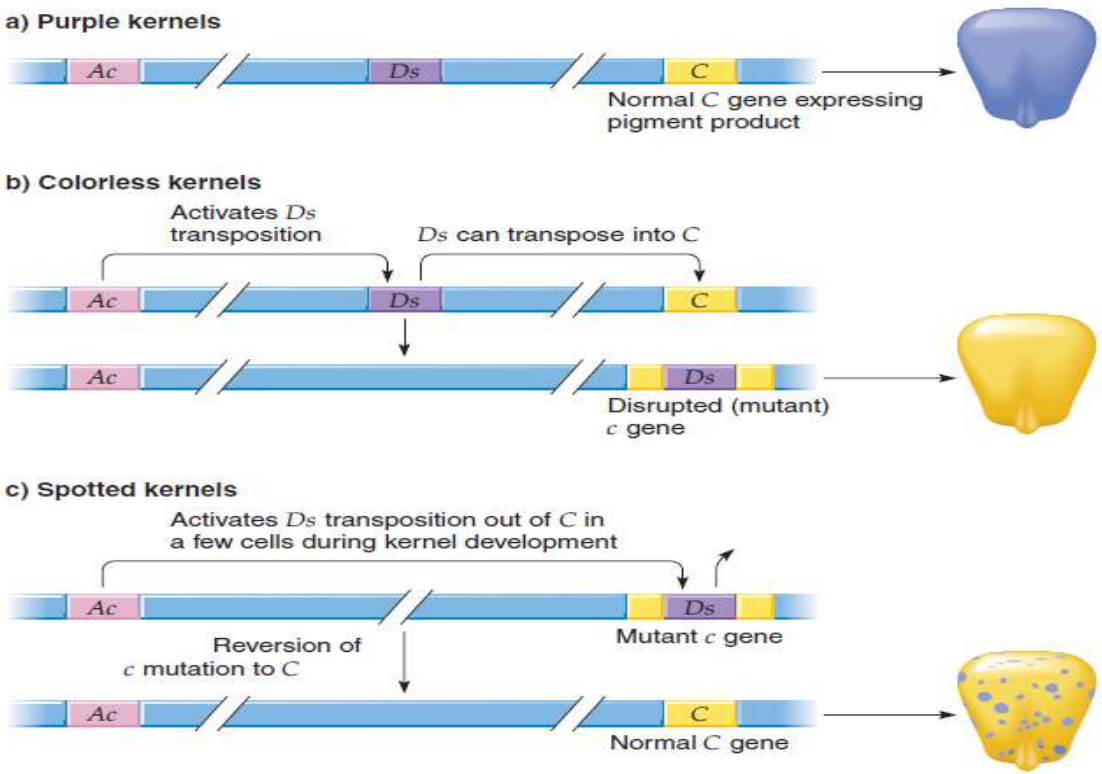


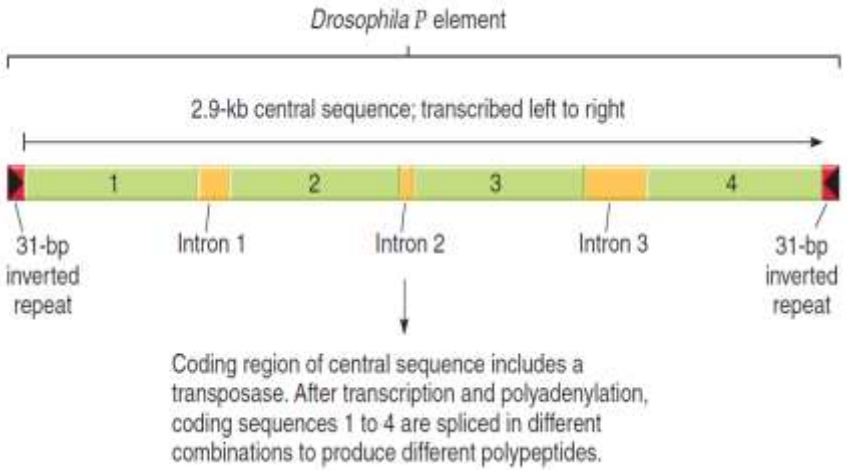
Figure 7.24
Kernel color and transposable element effects in corn. (a) Purple kernels result from the active *C* gene. (b) Colorless kernels can result when the *Ac* transposable element activates *Ds* transposition and *Ds* inserts into *C*, producing a mutation. (c) Spotted kernels result from reversion of the *c* mutation during kernel development when *Ac* activates *Ds* transposition out of the *C* gene.

Source: Peter J Russell, iGenetics A molecular approach, 3rd edition, Page no.157

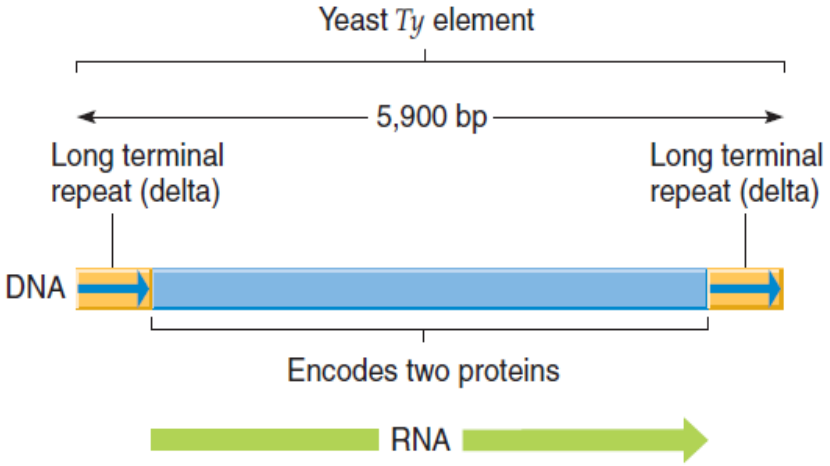
Rashmi M G

Transposable elements in Eukaryotes

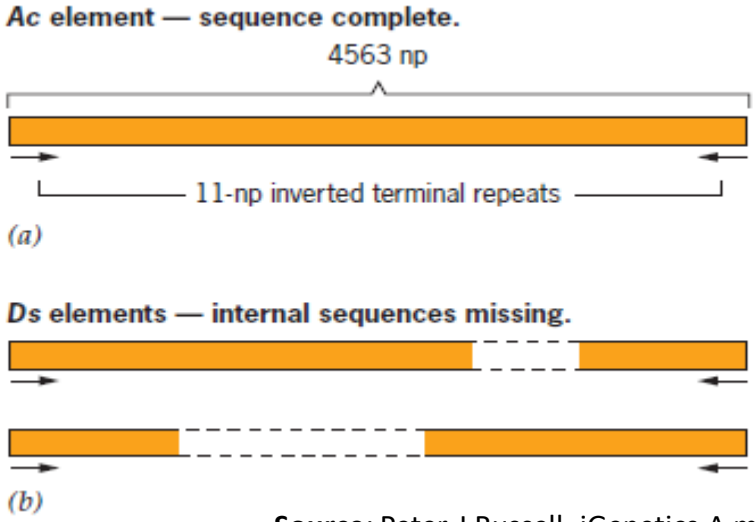
1. In Drosophila- **P elements**


Drosophila P element
2.9-kb central sequence; transcribed left to right
1
2
3
4
31-bp inverted repeat
Intron 1
Intron 2
Intron 3
31-bp inverted repeat
Coding region of central sequence includes a transposase. After transcription and polyadenylation, coding sequences 1 to 4 are spliced in different combinations to produce different polypeptides.

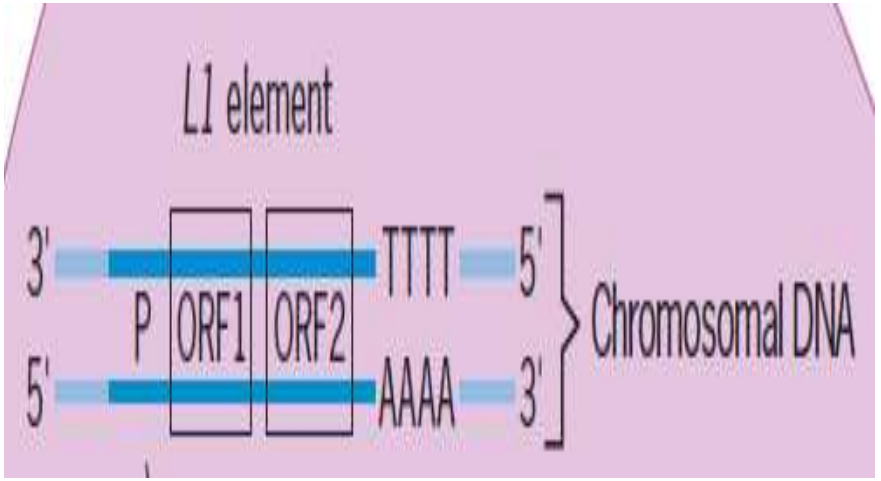
2. In Yeast- **Ty elements**



3. In corn- **Ac Ds elements**



4. In humans-**LINES/ L1 elements**



Source: Peter J Russell, iGenetics A molecular approach, 3rd edition, Page no.151-160

Rashmi M G

DNA repair mechanisms

Rashmi M G

DNA repair mechanisms

Genetic stability is achieved through,

- Accurate mechanism for replication DNA
- Repair mechanism to many accidental lesions

Immediate correction in DNA by set of process= **DNA repair**

4 Categories of DNA repair system

Direct repair
Acts directly on damaged nucleotides

Excision repair
Excision of segment of polynucleotide containing damaged site

Mismatch repair
Detects mismatch occur in DNA replication

Recombination repair
Process of filling gap in one strand of dsDNA

Direct repair

- Acts directly on damaged nucleotides
- Converts each one back to its original structure
- Ex. UV radiation mediated damages
  Repair of O^6 Methyl guanine

Pyrimidine dimer –repaired by light dependent direct system (Photoreactivation)

In E coli Process of Photoreactivation involves enzyme **DNA photolyase**

On induction of light of 300-500nm Enzyme binds to pyrimidine dimer and converted back to original monomeric nucleotide

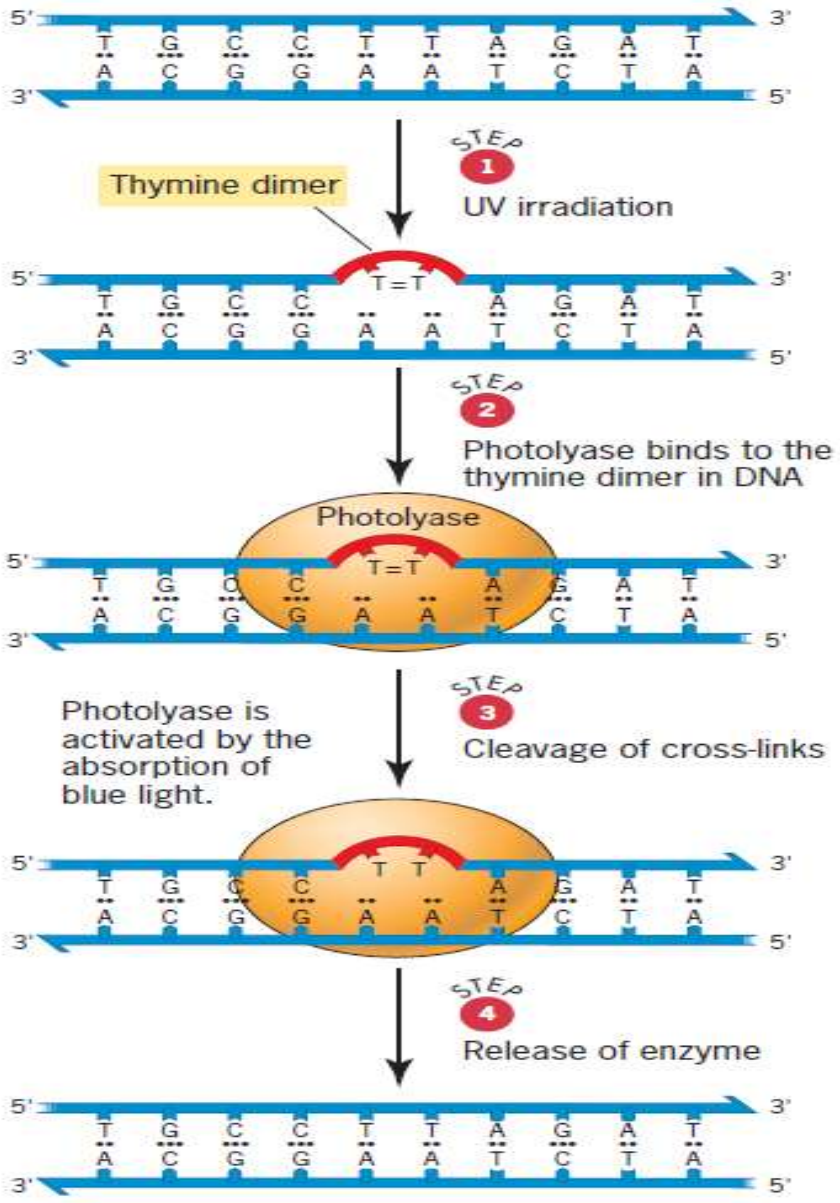


FIGURE 13.25 Cleavage of thymine dimer cross-links by light-activated photolyase. The arrows indicate the opposite polarity of the complementary strands of DNA.

Source: Snustad, Simmons (2012), Principles of genetics, John Wiley& Sons, Inc, Sixth edition, Page no. 348

Rashmi M G

Excision repair

- Excision of segment of polynucleotide containing damaged site
- Resynthesis of correct nucleotide sequence by DNA polymerase

2 types of Excision repair

Base Excision repair

Removal of damaged nucleotide base

Excision of short piece of polynucleotide

Resynthesis with DNA polymerase

Used to repair:

Alkylation damage

Deamination damage

DNA

Nucleotide Excision repair

Breaking phosphodiester bond on either side of lesion

Excision of oligonucleotide

Excision forms gap

Gap filled by repair synthesis

Ligase- seals the break

Base excision repair

Key enzyme- **DNA Glycosylase**

- Cleaves N-Glycosidic bond
- Liberates altered base
- Generates Apurinic/Apyrimidinic site (AP site)

AP endonuclease repair pathway:

- Introduces chain breaks by cleaving the phosphodiester bond at AP site
- Bond cleavage initiates

Excision repair process done by 3 enzymes:

- Exonuclease
- DNA polymerase I
- DNA Ligase

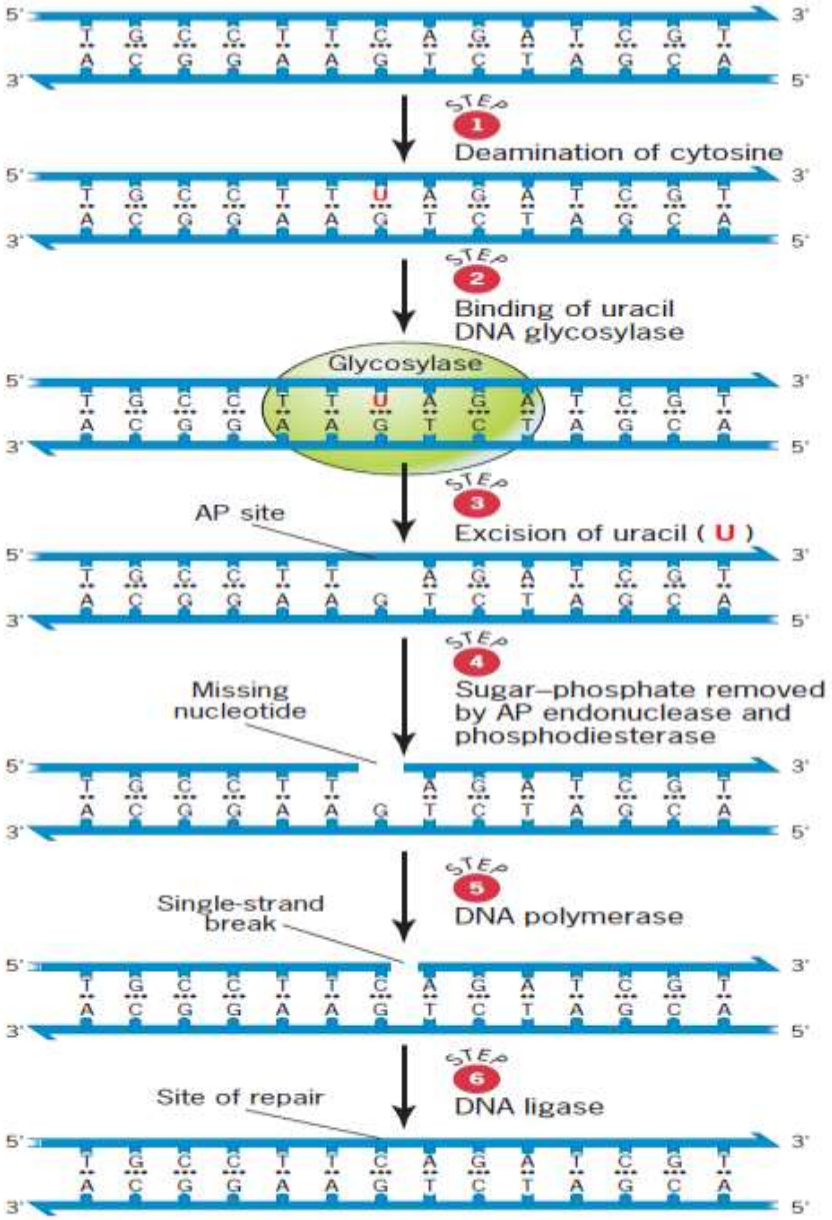


FIGURE 13.26 Repair of DNA by the base excision pathway. Base excision repair may be initiated by any one of several different DNA glycosylases. In the example shown, uracil DNA glycosylase starts the repair process.

Source: Snustad, Simmons (2012), Principles of genetics, John Wiley& Sons, Inc, Sixth edition, Page no. 349

Nucleotide excision repair

Key enzyme- **ABC excinuclease**

Ex. uvr system in E coli

ABC excinuclease binds to DNA at site of lesion

The key enzyme has 3 subunits:

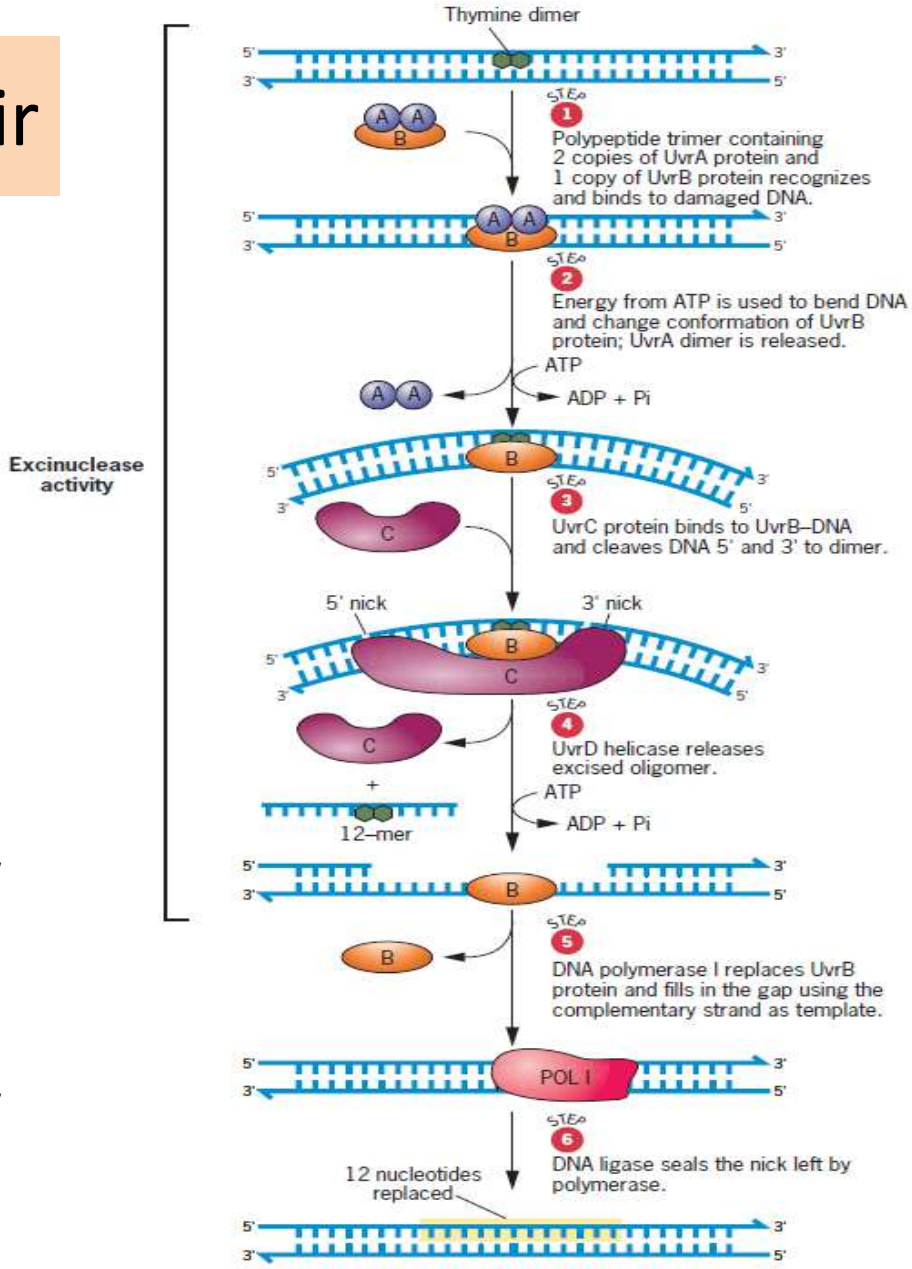
- uvr A- Recognize damage
- uvr B- Binds to damaged site
- uvr C- Forms uvr BC dimer and cleaves Damaged Strand

uvr D-helicase- helps to unwind DNA

In humans, **Xeroderma pigmentosum** and **Cockayne syndrome** is caused by genetically defective nucleotide excision repair

Inability of skin cells to repair UV induced DNA lesions (Pyrimidine Dimer)

Autosomal recessive condition, extremely sensitive to sunlight


Thymine dimer
Excinuclease activity
STEP 1
Polypeptide trimer containing 2 copies of UvrA protein and 1 copy of UvrB protein recognizes and binds to damaged DNA.
STEP 2
Energy from ATP is used to bend DNA and change conformation of UvrB protein; UvrA dimer is released.
ATP
ADP + Pi
STEP 3
UvrC protein binds to UvrB–DNA and cleaves DNA 5' and 3' to dimer.
5' nick
3' nick
STEP 4
UvrD helicase releases excised oligomer.
12–mer
ATP
ADP + Pi
STEP 5
DNA polymerase I replaces UvrB protein and fills in the gap using the complementary strand as template.
POL I
STEP 6
DNA ligase seals the nick left by polymerase.
12 nucleotides replaced

■ **FIGURE 13.27** Repair of DNA by the nucleotide excision pathway in *E. coli*. The excinuclease (excision nuclease) activity requires the products of three genes—*uvrA*, *uvrB*, and *uvrC*. Nucleotide excision occurs by a similar pathway in humans, except that many more proteins are involved and a 24-to-32-nucleotide-long oligomer is excised.

Source: Snustad, Simmons (2012), Principles of genetics, John Wiley& Sons, Inc, Sixth edition, Page no. 350

Mismatch repair

Key enzyme- **DNA helicase II** and **exonuclease I**

Proteins **– Mut H**, **Mut L**, **Mut S**

Recognize mismatched base pairs, determine which base in mismatch is incorrect one, Excise incorrect base and carry out repair synthesis

Mut H- recognize sequence GATC

Mut L- Function is not known

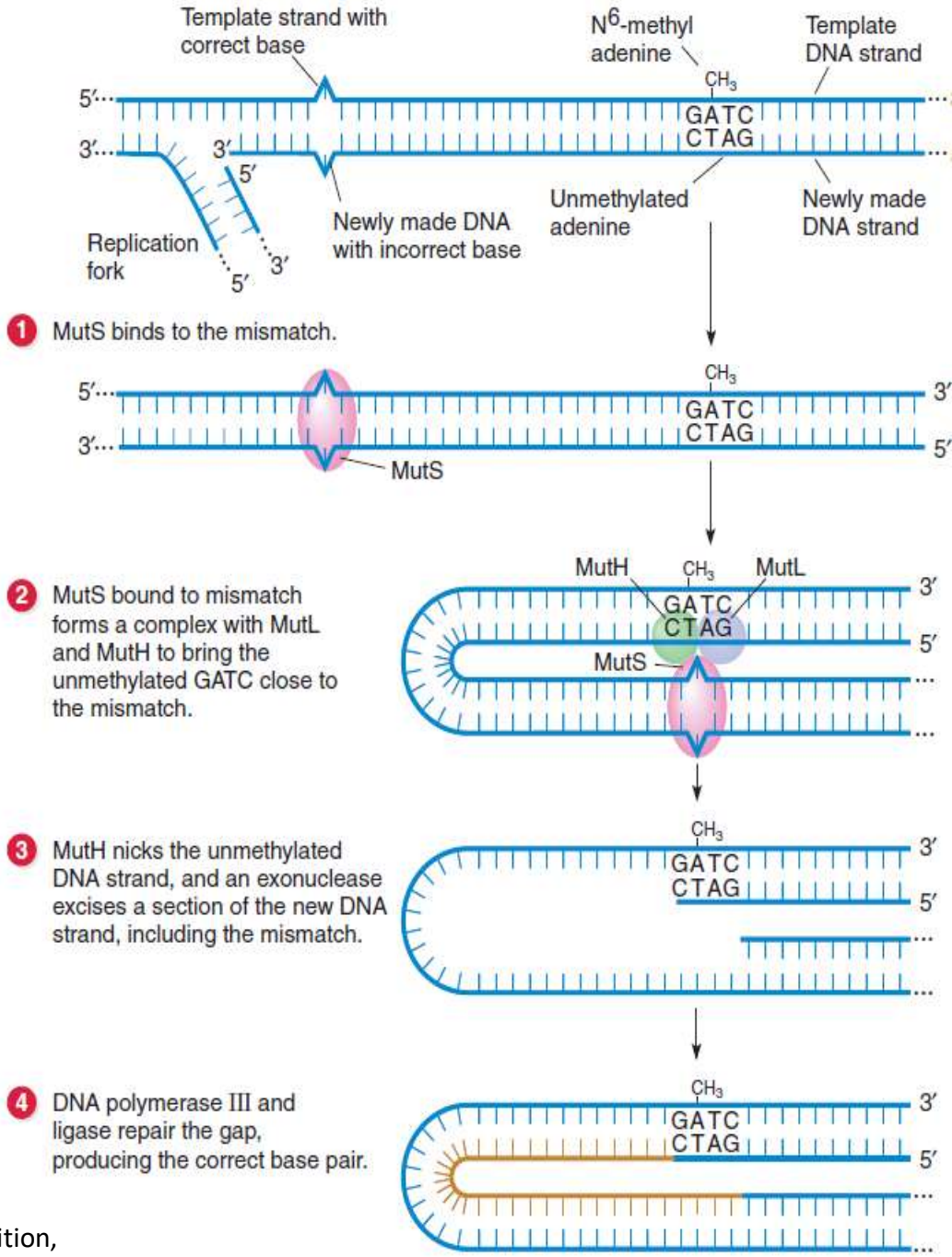
Mut S- Recognize mismatch

Mut H protein- cleaves unmethylated strand on 5’ side of G in GATC sequence

DNA helicase II + Exonuclease I- Removes segment of new strand between cleavage site and point just beyond the mismatch

Gap- Filled by DNA polymerase

Nick- sealed by DNA Ligase


Template strand with correct base
N6-methyl adenine
Template DNA strand
CH3
GATC
CTAG
Unmethylated adenine
Newly made DNA strand
Newly made DNA with incorrect base
Replication fork
1 MutS binds to the mismatch.
MutS
2 MutS bound to mismatch forms a complex with MutL and MutH to bring the unmethylated GATC close to the mismatch.
MutH
MutL
3 MutH nicks the unmethylated DNA strand, and an exonuclease excises a section of the new DNA strand, including the mismatch.
4 DNA polymerase III and ligase repair the gap, producing the correct base pair.

Source: Peter J Russell, iGenetics A molecular approach, 3rd edition, Page no.149

Rashmi M G

Recombinational repair

Post replication repair method

Ex. Pyrimidine dimer- on one strand of double helix

DNA replicated

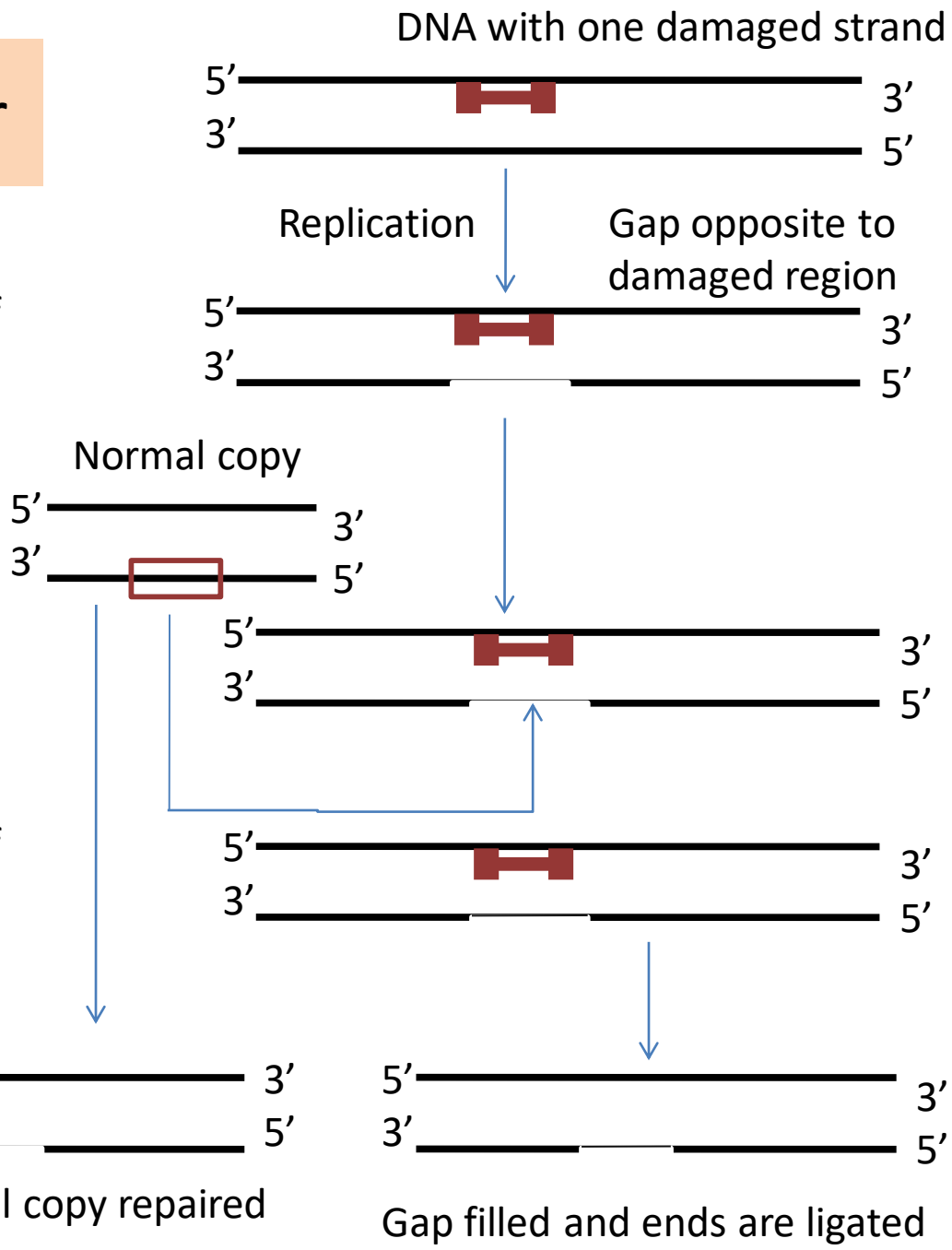
Dimer prevents damaged site from acting as template

Gap in newly synthesized strand

Other parental strand- forms normal complementary strand

Repair mechanism:

Gap opposite the damaged site- filled by taking the homologous single strand of DNA from Normal Duplex



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Repair of double strand DNA break

Ionizing radiation
Oxidizing agents
Replication errors

→ Cause **double strand break in DNA**

→ If left unrepaired , leads to breakdown of chromosome into small fragments

2 Repair pathways

NHEJ pathway

- Cell can repair strand breaks without the needs for a homologous template
- Leads to insertions/ deletions of nucleotides (Indels) at site of repair
- Highly efficient pathway
- Error- prone pathway
- Present throughout cell cycle
- Common in G_0 and G_1 Phase
- Emergency solution to repair double strand break

HDR pathway

- Uses homologous DNA as a template
- Homologous sister chromatids is available as a template for repair
- Error free DNA repair
- Predominant in S and G_2 phase

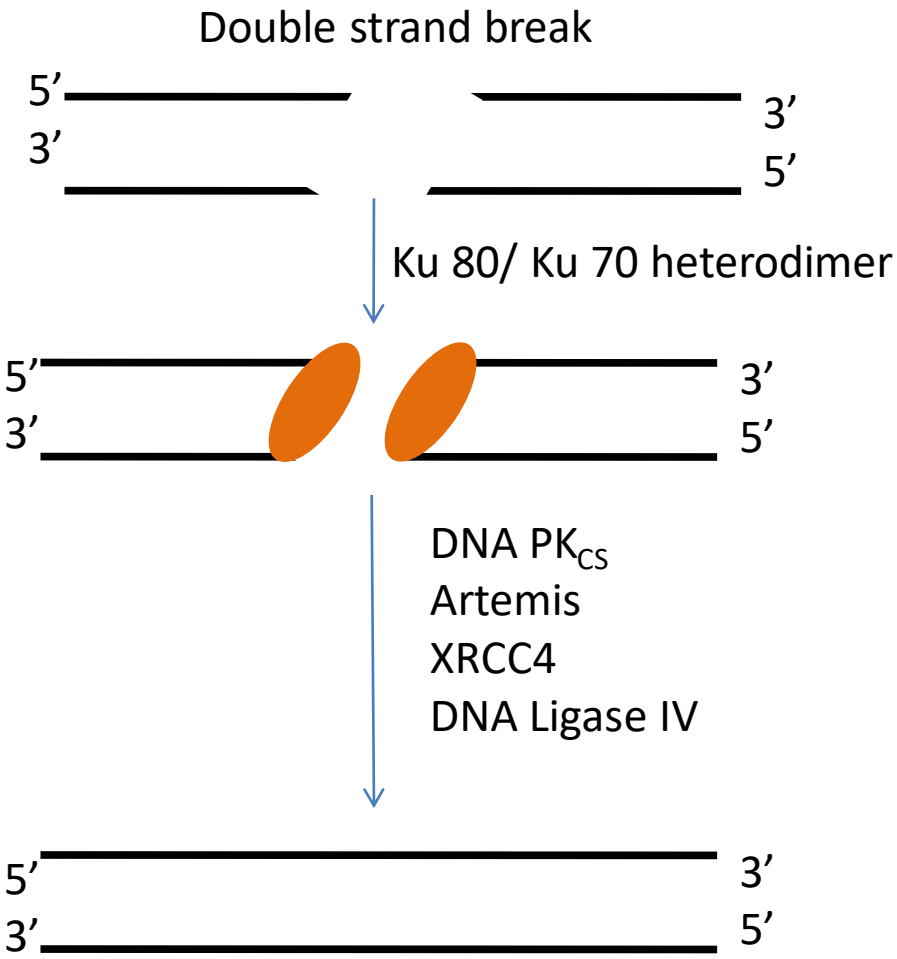
Non homologous end joining pathway (NHEJ)

Broken ends- juxtaposed and rejoined by DNA Ligation

Loss of 1 / more nucleotides at site of joining

Mechanism:

1. Recognition of broken ends by heterodimer Ku70 and Ku 80
2. Ku proteins- DNA dependent protein kinase (DNA PK_{CS})
3. Phosphorylation triggers recruitment of end processing factors (Nuclease Artemis)
4. Trim DNA ends makes compatible for ligation
5. Final joining by DNA Ligase IV andXRCC4



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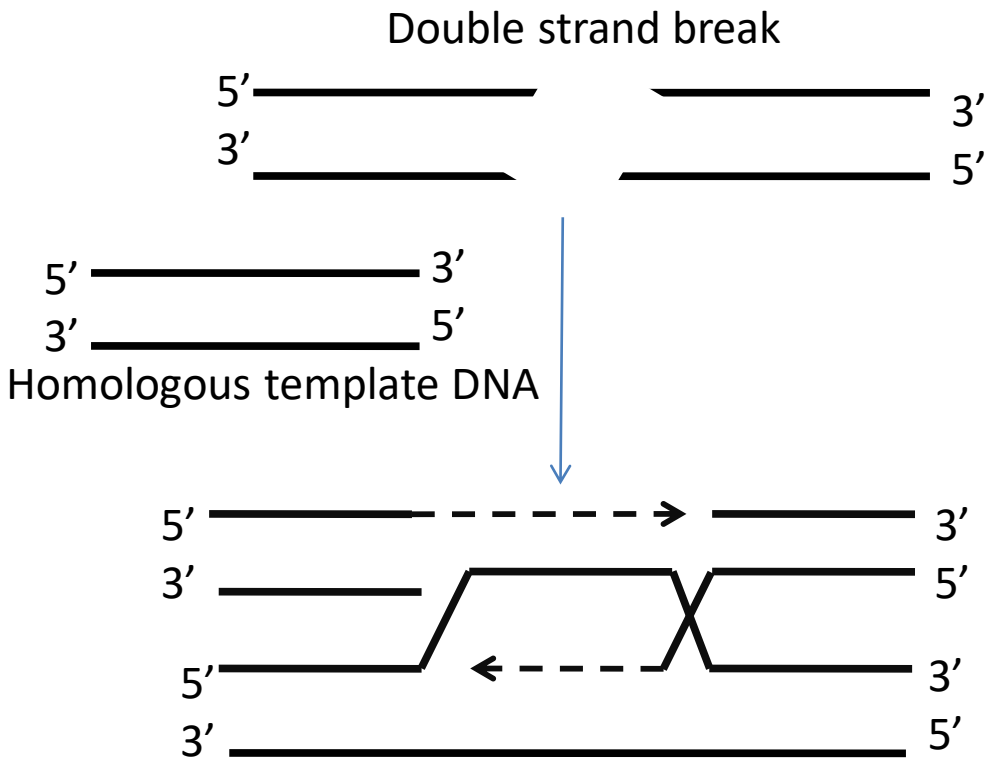
Homology directed repair pathway (HDR)



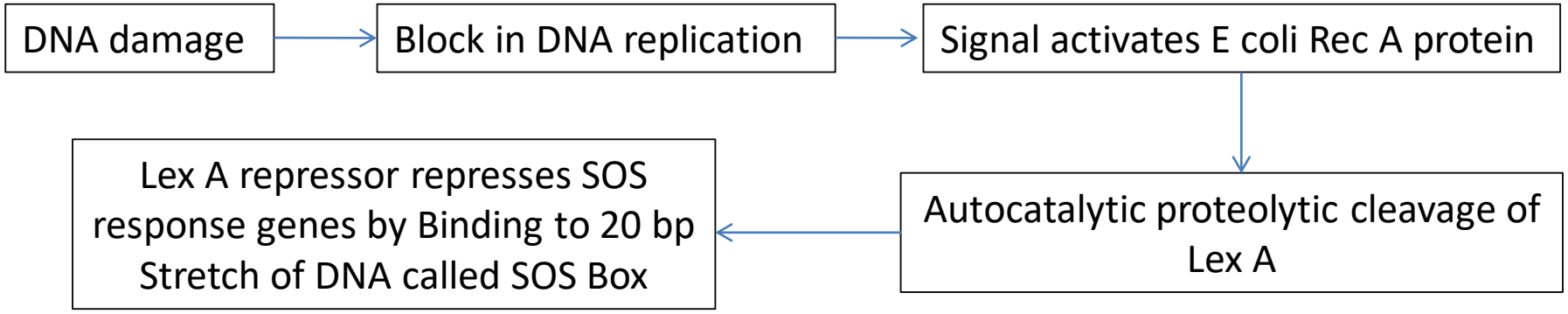
SOS response

Cells have mechanism – enables to synthesize **DNA repair enzymes**
As emergency response- to severe DNA damage
Ex. SOS response in E coli
Aids in survival of organism by affecting expression of proteins that are involved in cell division, replication, recombination and excision repair
Key regulatory elements:

- Repressor- **Lex A**
- **Rec A** protein

Repressor –**Lex A**

- Acts as co protease stimulates protease activity
- Promotes base pairing between single strand of DNA and its complement


DNA damage
Block in DNA replication
Signal activates E coli Rec A protein
Autocatalytic proteolytic cleavage of Lex A
Lex A repressor represses SOS response genes by Binding to 20 bp Stretch of DNA called SOS Box

Summary

Nucleotide excision repair • Damaged nucleotides are removed by breaking phosphodiester bond • Deficiency of enzyme in human increases risk of skin cancer • DNA Ligase catalyze final reaction	**Base excision repair** • Damages nucleotide are removed by breaking Phosphodiester bond • DNA Ligase catalyze final reaction
Photoreactivation • Free radical mechanism is involved • Key enzyme contains bound Folate cofactor • No base/ nucleotides are removed from DNA	**Alkyl transferase repair** • Repair enzyme functions only once • No base/ nucleotides are removed from DNA
Mismatch repair • Process begins upto 1 kbp away from repair site • DNA Ligase catalyze final reaction	**SOS repair** • Rec A participated • System is responsible for error prone replication • DNA Ligase catalyze final reaction